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# THE JOURNAL OF EXPERIMENTAL MEDICINE

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# THE JOURNAL

OF

# EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

VOLUME TWENTY-NINTH

WITH THIRTY-EIGHT PLATES AND NINETY FIGURES IN THE TEXT




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# Herbert Douglas Taylor

Born 1889 Died 1918

*At the meeting of the Board of Scientific Directors of The Rockefeller Institute for Medical Research, held on October 19th, 1918, the following note was placed on the Minutes:*

*"The Board of Directors of The Rockefeller Institute records with sorrow the death, on October 7th, 1918, in the service of his country, of Lieutenant Herbert Douglas Taylor of the Medical Corps of the United States Army. His notable contributions to science, his unswerving devotion to his chosen service for the general welfare, his inspiring idealism, and his genial comradeship won for him, in no common measure, the esteem, friendship, and love of his fellow workers of the Institute."*

*Lieutenant Taylor's short career in scientific research was marked by such energy, enthusiasm, and clearness of vision as were a real inspiration to his fellow workers. During his three and one-half years at The Rockefeller Institute his studies dealt mainly with problems associated with cancer and tuberculosis. Soon after the United States entered the war he offered his services and was appointed First Lieutenant in the Medical Corps of the Army, and assigned as instructor of medical officers in the War Demonstration Hospital and in the Laboratory Courses of the Institute. He threw himself into the new work with characteristic energy. Not content with teaching and the routine work of the War Demonstration Hospital, he started extensive investigations of the action of antiseptics on infected wounds and contributed important papers on this subject. At the beginning of the epidemic of influenza he took up certain phases of this problem to which he gave himself without reserve. In the course of his studies he contracted influenza and died after a short illness.*

*This number of The Journal of Experimental Medicine, contributed by the Department of which Lieutenant Taylor was a member, was prepared with his enthusiastic cooperation. It contains much of his own completed work. His fellow workers offer it as a memorial to a spirit and an example that will not die.*

All the studies in this issue have been carried out in the section of the Division of Pathology and Bacteriology of which Dr. James B. Murphy has charge.

# EFFECT OF DRY HEAT ON THE BLOOD COUNT IN ANIMALS.

## III. Studies on Lymphoid Activity.

By JAMES B. MURPHY, M.D., and ERNEST STURM.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 1 to 3.

(Received for publication, October 17, 1918.)

The study of the function of the lymphoid tissue has, in the past, been productive of few leads of importance, due undoubtedly to the lack of methods for approaching the subject. It has not been possible, owing to the wide distribution of the lymphoid tissue throughout the body, to employ the method so productive in the study of deficiency of function in the glands of internal secretion brought about by partial or complete removal. Extensive investigations on animals and man after removal of the spleen, the chief lymphoid depot, has added little to our understanding of the function of this type of tissue. The loss under these conditions is so rapidly compensated for by hyperplasia of the lymphoid tissues that a diminished function cannot be detected. By utilizing the well known fact that lymphoid tissue is extremely sensitive to x-rays, we were able to develop a method by which practically the entire lymphoid tissue of the body can be destroyed with a minimum destruction of other tissue and slight, if any, effect on the general health of the animal.[1] The method consisted of small, repeated doses of x-rays distributed over from 7 to 21 days, depending on the size and resistance of the animal. The experiments carried out on the delymphocytized animals led to the following results: (1) destruction of the mechanism of resistance against implants of foreign tissue;[1] (2) lowered resistance to inoculated cancer grafts;[2] (3) destruction of established immunity to cancer;[3]

[1] Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[3] Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1. Mottram, J. C., and Russ, S., *Proc. Roy. Soc. London, Series B*, 1917–18, xc, 1.

(4) lowered resistance to tuberculosis in mice,[4] guinea pigs,[5] and monkeys;[6] (5) lowered resistance to poliomyelitis in monkeys.[7]

From the experimental side we have been able to get suggestive results after the stimulation or apparent increased activity of the lymphoid tissue. The chick embryo, normally with no resistance against transplants of heteroplastic tissue, can be rendered resistant by suitable transplants of adult lymphoid tissue.[8] A single small dose of x-rays, sufficient to stimulate somewhat the circulating lymphocytes, increased the resistance of mice to autotransplants of spontaneous cancers.[9] Mice with high lymphoid counts resulting from splenectomy or from the reaction engendered in a cancer-immune animal after inoculation with a cancer graft show a marked increase in resistance to bovine tuberculosis.[4,10] All the results given above suggest, rather than prove, that the lymphocyte is an active agent in these varied processes. It seems necessary to test more completely the effect of stimulation by other means.

Many methods have been tested to produce an extensive and enduring increase in the lymphocyte and lymphoid tissue. Among these were the use of a number of drugs and dyes, ultra-violet light, and sunlight.[11] Except in the cases of the two latter agents, the results were unsatisfactory. Either the increase was too slight to be of experimental value, or not of long enough duration, or the incidental disturbance to the general health of the animal was too great. One highly promising method has been found; namely, the use of intense dry heat.

## *Method.*

The source of the heat in these experiments was an electric bulb of frosted glass, giving the maximum heat for the amount of light generated. The method of application varied with the size of the

[4] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.
[5] Morton, J. J., *J. Exp. Med.*, 1916, xxiv, 419.
[6] Taylor, H. D., unpublished observation.
[7] Amoss, H. L., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 115.
[8] Murphy, Jas. B., *J. Exp. Med.*, 1914, xix, 513.
[9] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.
[10] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.
[11] Taylor, H. D., *J. Exp. Med.*, 1919, xxix, 41.

animal and its ability to stand the higher temperatures. For mice an ordinary iron tripod 9 inches high and $5\frac{1}{2}$ inches at the base was covered on the sides with cardboard. A space was left at the bottom for free circulation of air. This was placed over an electric "Sunbeam" heating lamp of 110 volts, 80 watts direct current. A small circular cage of the same size as the opening was placed on top of the tripod. This was made of galvanized iron with a wire mesh bottom ($\frac{1}{4}$ inch mesh). The distance from the top of the lamp to the bottom of the cage was 3 inches. A thermometer was introduced parallel to and about $\frac{1}{4}$ inch below the bottom of the cage, directly over the light. After the light was turned on, the temperature was allowed to rise to 55°C. before the mouse was put into the cage. The animal was allowed to remain 5 minutes, this being about the time required for the temperature to rise from 55–65°C. If the temperature rose too rapidly it was found advisable to shut off the light until the thermometer registered between 57° and 59°C. and then allow it to increase again to 65°. For mice the temperature should never exceed 65°, as they can survive only 1 or 2 minutes at a higher temperature than this. A small piece of blotting paper was placed in the bottom of the cage just large enough for the mouse to rest on. In our experiments the loss was less than $\frac{1}{2}$ of 1 per cent, and only occasionally were burns encountered.

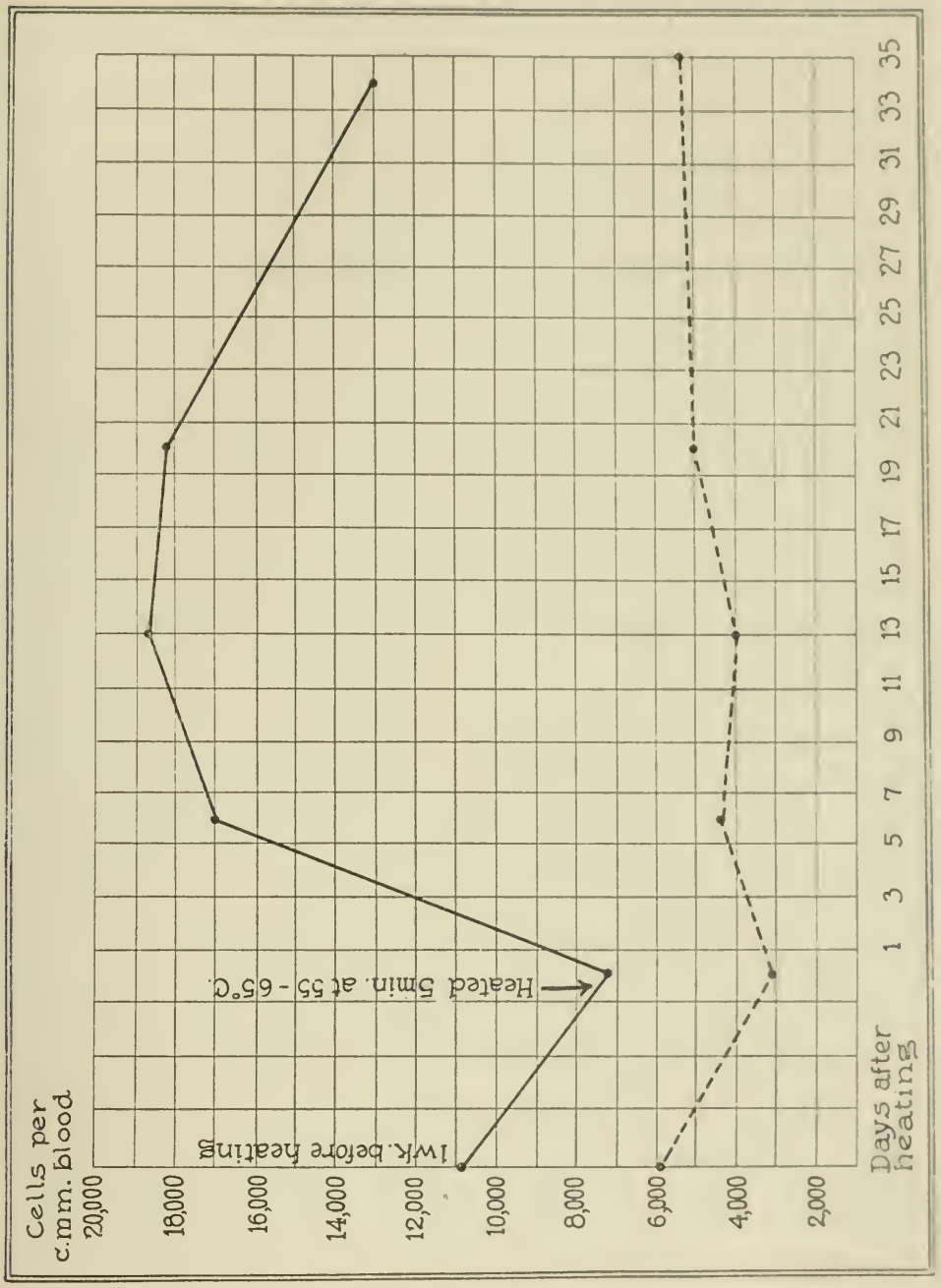
For heating larger animals, such as rats or guinea pigs, a wooden box 9 by 6 inches and $4\frac{1}{2}$ inches high was used with an electric carbon heating lamp 25 volts, 100 watts direct current, suspended directly over it. This lamp was surrounded by a metal reflecting cup. The distance from the lamp to the top of the box was 10 inches. The thermometer was placed at the level of the body of the animal. Rats and guinea pigs were found much more susceptible to higher temperatures than mice. A temperature of 60°C. or more caused extreme discomfort. The animals can, however, stand a more prolonged exposure at the lower temperature than can mice. For this reason it was found advisable to reduce the maximum temperature and increase the time of exposure for these animals.

## *Effect of Dry Heat on the Leucocytes in Mice.*

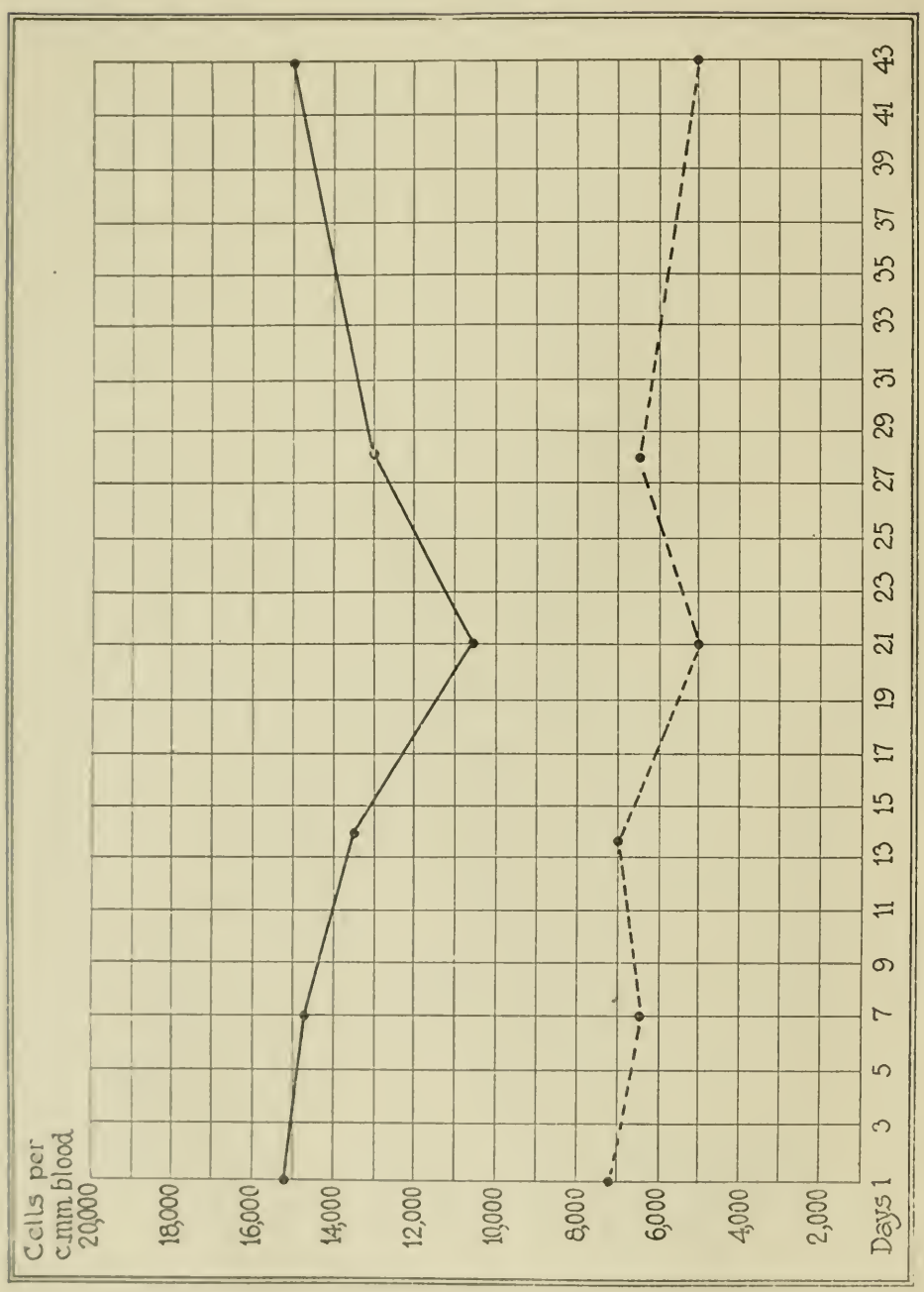
*Experiment 1.*—Total and differential leucocyte counts were made on fifteen healthy mice of about the same age and size. 1 week later ten of these animals were heated over an electric lamp at a temperature ranging from 55–65°C. for 5 minutes. White cell and differential counts were made immediately after this procedure, and counts were also made on five remaining animals as controls. All the fifteen animals were counted again, 7, 14, and 21 days after heating. On charting the results of the counts it was found that immediately after heat exposure there was a uniform decrease in both principal types of white cells, the lymphocytes and the polymorphonuclear leucocytes, which averaged about 3,000 cells per c.mm. of blood. The count on the five unheated mice showed no appreciable change. 7 days after heating it was found that there was an increase of from 5,000 to 10,000 cells per c.mm., this increase being almost entirely in the lymphoid group. The polymorphonuclear leucocytes remained lower than they were in the original count. The blood picture in the five control mice was similar to that at the previous determinations. 14 days after heating, the lymphocytes had increased to a figure above the original count in some of the heated animals to the extent of from 12,000 to 14,000 cells per c.mm. of blood, while the polymorphonuclear leucocytes still remained below the original determination in practically all the animals. The control animals showed no change beyond that well within the bounds of experimental error. 21 days after heating, the lymphocytes, although several thousand cells above the original count, had fallen off somewhat from that of the previous week. The polymorphonuclear cells at this point showed a tendency to rise. These results are graphically recorded in Text-fig. 1 for the heated animals and Text-fig. 2 for the controls.[12]

*Experiment 2.*—White blood counts were made on ten healthy mice of approximately the same age and size. A week later five of these (Group I) were heated for 5 minutes at a temperature of from 55–65°C. Immediately afterward blood counts were made on these and the five unheated animals (Group II). A week later the control mice (Group II) were heated in the same manner as that employed with Group I the previous week. Counts were made on both groups at this period and repeated 7 and 21 days later. Group I showed an average decrease of 2,500 lymphocytes per c.mm. of blood after heating, while the polymorphonuclear leucocytes dropped more than 6,000 cells. Group II, which was used as the control up to this point, showed little change in the blood picture. Counts a week later on Group I, 1 week after heating, showed an average increase of over 6,000 lymphocytes per c.mm. above the normal level. The polymorphonuclear leucocytes had recovered somewhat from the effect of the heat, but were still below the normal. Group II at this time, after having been counted twice with

[12] From past experience it was found that if counts were made more often than once a week it caused too great a fluctuation in the count.

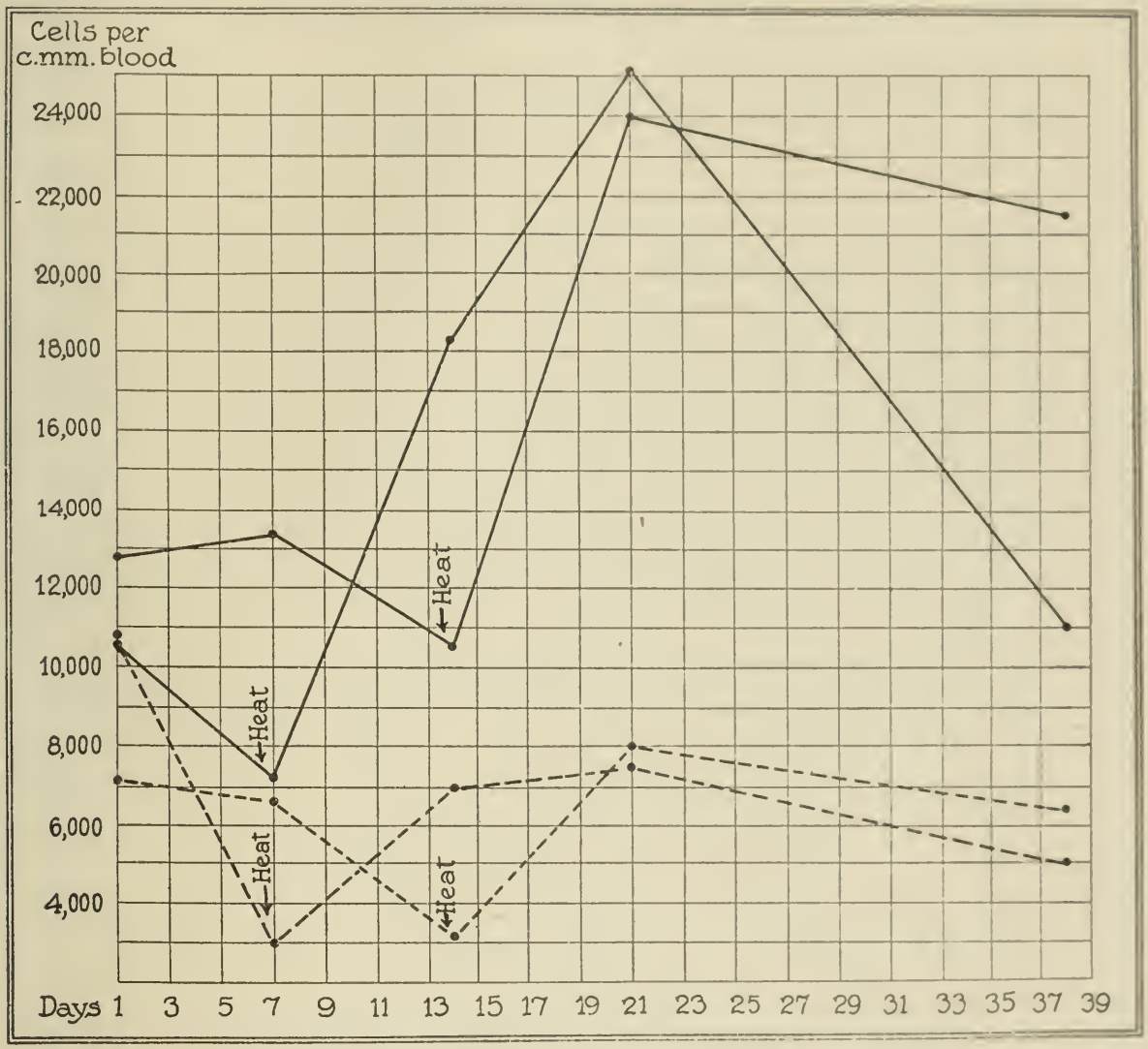



TEXT-FIG. 1. Composite curve of the white blood cell counts on ten mice before and after exposure to dry heat. ————Lymphocytes. .....Polymorphonuclear leucocytes.




TEXT-FIG. 2. Composite curve of the white blood cell counts on five normal mice of the same strain as those in Text-fig. 1. The animals were kept under the same conditions, and counts were made at the same time. ——Lymphocytes. .....Polymorphonuclear leucocytes.

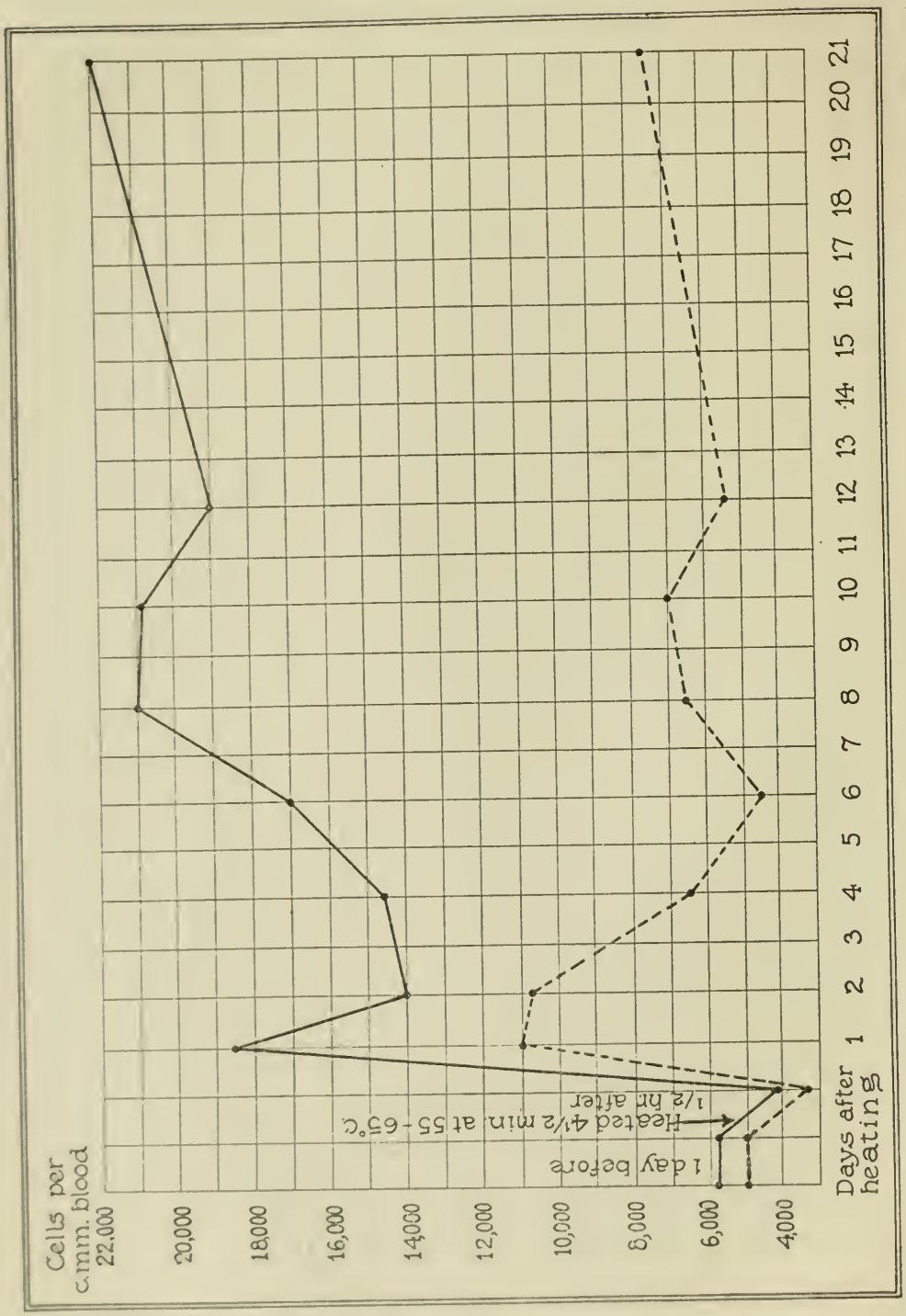
slight variation, showed the same characteristic drop in the blood count after heat was applied. After another 7 day interval the counts on Group I, 14 days after heating, showed an increase of 14,000 lymphocytes per c.mm. over the original count. Group II, 7 days after heating, showed an increase of 12,000 lymphocytes over the two previous counts. 14 days later Group I, 28 days




TEXT-FIG. 3. Composite curve of the white blood cell counts on two groups of mice, five in each group. One lot was heated a week after the first count, the others were heated 2 weeks after the first count with one intervening count. ————Lymphocytes. .....Polymorphonuclear leucocytes.

after heating, showed a count practically returned to its normal level, while Group II, 21 days after heating, was still several thousand cells above the normal count. This experiment is graphically illustrated in Text-fig. 3.

*Experiment 3.*—White blood counts were made on twenty healthy mice. 1 week later the entire twenty animals were heated for 5 minutes from 55–65°C.




TEXT-FIG. 4. Composite curve of the white blood cell counts on twenty healthy mice before and at intervals after heating. The first count represents the average for the whole lot. After heating they were counted in groups of five animals, the first group immediately after heating, the next 24 hours later, and then at intervals indicated on the chart. The last count represents the average of all twenty mice.

———Lymphocytes. .....Polymorphonuclear leucocytes.

and were then divided into four groups of five animals. Leucocyte counts were made on the first group immediately after heating, on the second 24 hours after heating, on the third 48 hours, and on the fourth 4 days after heating. Counts were made again on each group 6, 8, 10, and 12 days respectively after heating and again on all the animals 3 weeks after heating. The average of the counts for each day is shown graphically in Text-fig. 4, giving a continuous curve for the whole group.

*Experiments 4, 5, and 6.*—Three other experiments were carried out on mice in the same manner as those described above, with no variation in the result. These will not be given in detail.

In six experiments on mice it was found that after exposure of the animals to dry heat at 55–65°C. for 5 minutes there was a distinct fall in the total white blood cell count, both the polymorphonuclear leucocytes and the lymphocytes. The polymorphonuclear leucocytes recovered rather slowly, while the lymphocytes increased so rapidly that by the 2nd week after heating the count often reached a point 200 to 300 per cent above the normal figure.

The rectal temperature of mice after exposure to heat as described in the above experiments varies considerably, as shown by Table I.

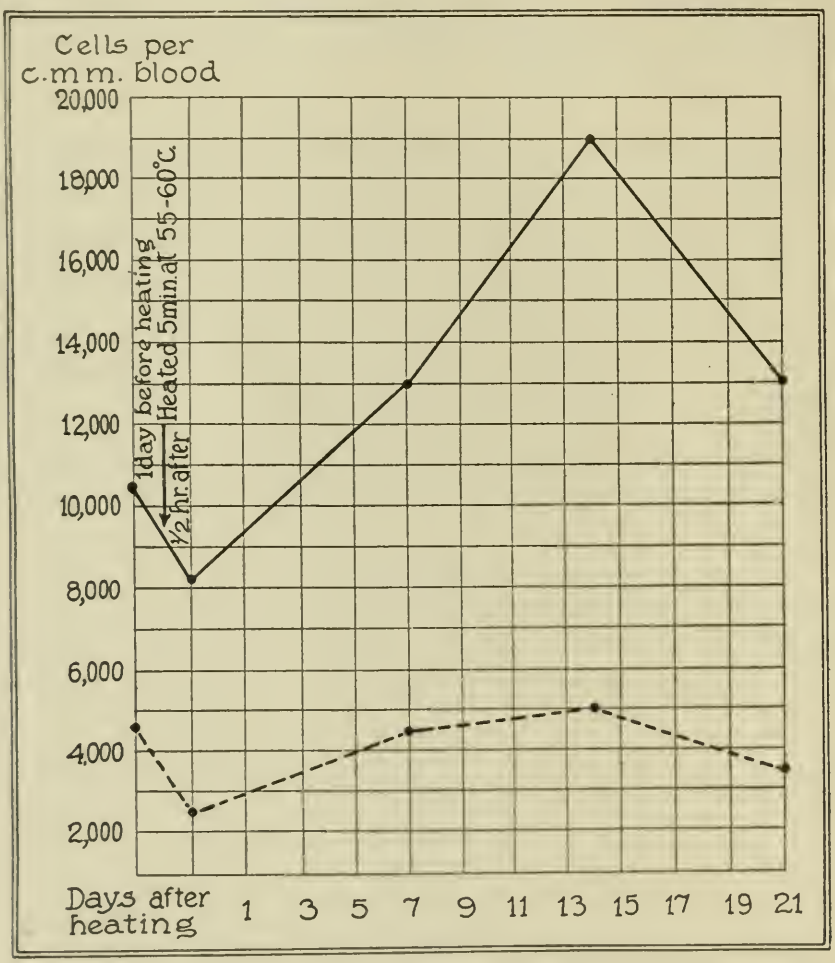
TABLE I.

| Experiment No. | Before heat. | Directly after heat. | ½ hr. after heat. | 1 hr. after heat. |
|---|---|---|---|---|
| | °C. | °C. | °C. | °C. |
| 1 | 36.2 | 39.1 | 38.3 | 37.7 |
| 2 | 36.7 | 39.0 | 37.2 | 37.0 |
| 3 | 36.6 | 39.7 | 37.1 | 35.1 |
| 4 | 36.0 | 41.6 | 35.7 | 35.2 |
| 5 | 37.2 | 40.8 | 38.3 | 37.7 |

*Morphology of Circulating Lymphocytes after Heating.*

No attempt has been made in this study to differentiate the large and small lymphocyte, as it is practically impossible to establish a satisfactory dividing line between the two types. In general it may be said that the larger type predominates in the earlier stages of the stimulation, while later the smaller ones increase. The cells of both types are for the most part normal, healthy looking lymphocytes, and do not differ in appearance from those observed in the

normal animal. At a period of from 6 to 10 days after heating it was noted that a proportion of the lymphocytes were in the process of what appeared to be amitotic division. Every stage (Figs. 1 and 2) of this division could be observed. In some of the more extreme cases there were found from three to five cells in one microscopic field (Fig. 3).

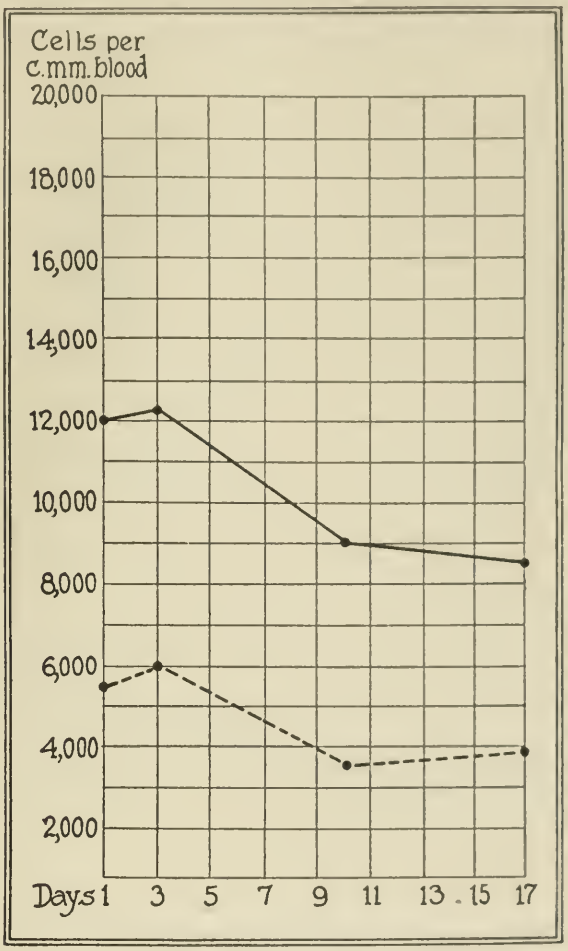



TEXT-FIG. 5. Composite curve of the white blood cell counts on five rats before and after heating. ————Lymphocytes. .....Polymorphonuclear leucocytes.

### *Effect of Heat on the Blood Picture in Rats and Guinea Pigs.*

*Experiment 7.*—Blood counts were made on ten healthy rats of about the same age and size. The following day five of these animals were heated for 5 minutes at 55–60°C., counts being made immediately afterward both on these and on five control animals. The heated rats showed a decrease in both types

of cells similar to that observed in mice. The controls showed no marked change in their count. 7 days later counts showed the lymphocytes in the heated animals to be markedly increased, with the polymorphonuclear cells practically returned to their normal level. Control animals at this period showed a slight falling off in the count. 14 days after heating there was an average rise of over

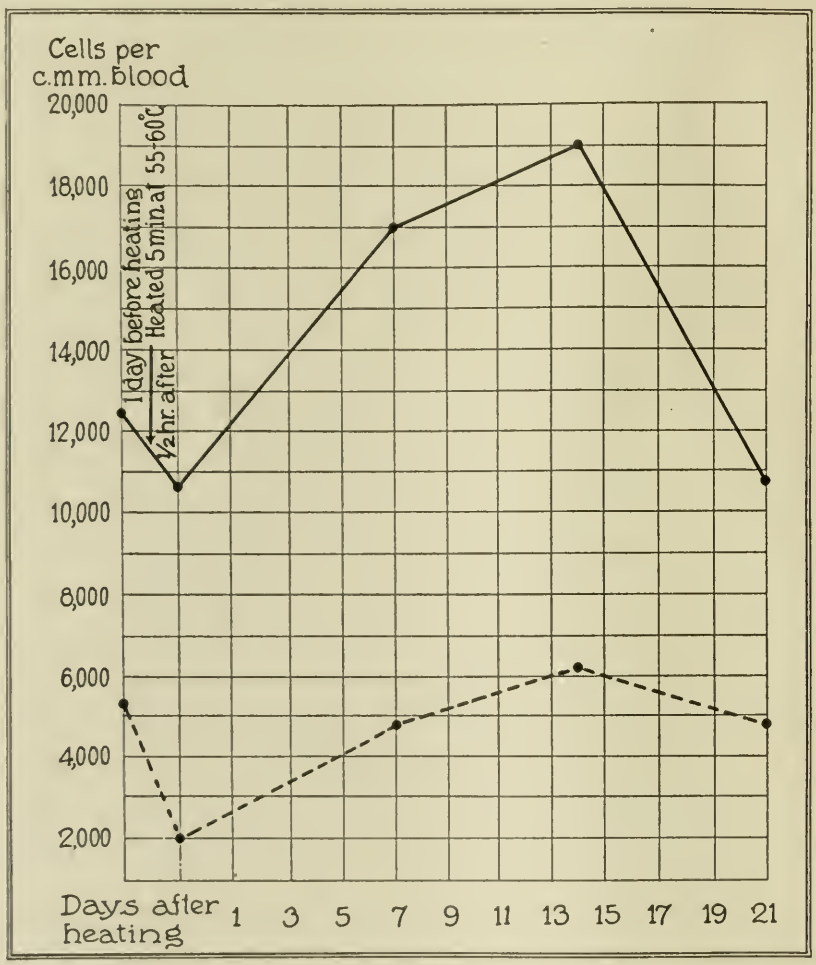



TEXT-FIG. 6. Composite curve of the white blood cell counts on five normal rats of the same lot as those in Text-fig. 5, kept under the same conditions, with counts made at the same time. ————Lymphocytes. .....Polymorphonuclear leucocytes.

8,000 cells per c.mm. of blood, while the polymorphonuclear count remained about the same. 21 days after heating, the lymphocytes showed a tendency to decrease, but still remained well above the normal level. Text-fig. 5 is the composite curve of the heated animals and Text-fig. 6 that of the controls. Text-fig. 7 shows the curve of a typical rat after heating.

Several other experiments of a similar character on rats gave identical results, the changes being similar in every way to those observed in mice.

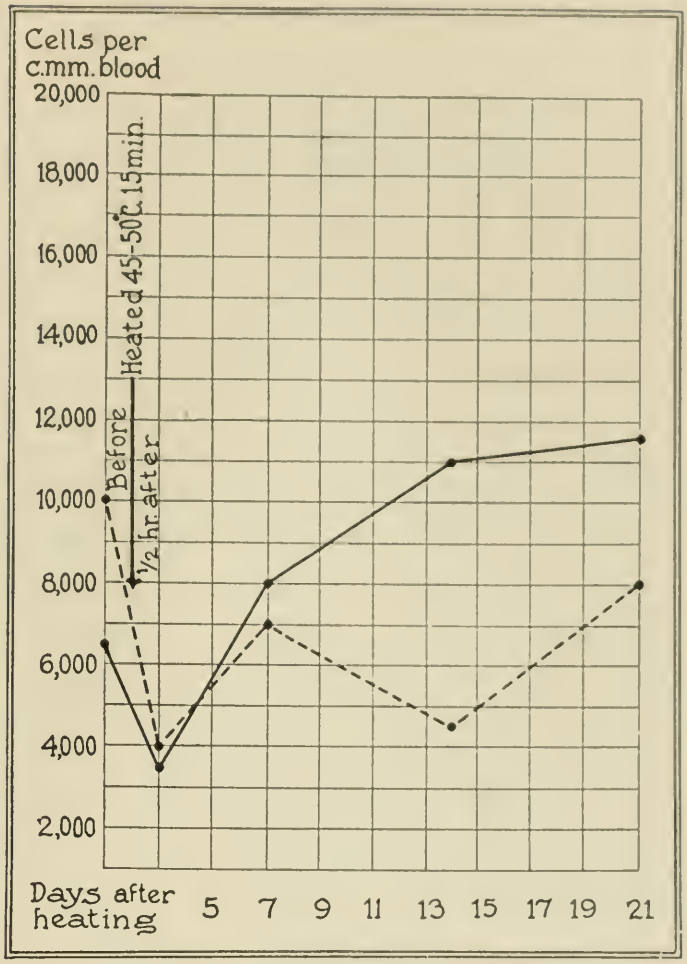
*Experiment 8.*—A total and differential leucocyte count was made on a fairly large guinea pig. The following day the animal was heated for 15 minutes at




TEXT-FIG. 7. Curve of the white blood cell counts of an individual rat after heating. ————Lymphocytes. .....Polymorphonuclear leucocytes.

45–50°C. and a count made shortly afterward. The lymphocytes showed only a slight decrease, while the polymorphonuclear leucocytes were reduced by 6,000 cells per c.mm. After this, counts were made at 7 day intervals for 3 weeks. They showed a steady increase in the lymphocytes, while the polymorphonuclears slowly regained their normal level and remained at that point (Text-fig. 8).

Several other experiments on guinea pigs gave identical results.




TEXT-FIG. 8. The white blood cell counts of the guinea pig after exposure to heat. ————Lymphocytes. .....Polymorphonuclear leucocytes.

## DISCUSSION.

Some years ago Wickline[13] made a study of the blood cells in American troops incidental to the complete physical examination made at intervals during residence in the Philippines. He showed that there was a marked increase in the relative and absolute number of mononuclear blood units, the increase being at the expense of the polymorphonuclear leucocytes. There was no marked change in the total white cell count. Chamberlain,[14] later, incidental to the study of the Arneth count in the tropics, confirmed and extended

[13] Wickline, W. A., *Mil. Surg.*, 1908, xxiii, 282.
[14] Chamberlain, W. P., *Mil. Surg.*, 1909, xxv, 48.

this interesting observation. There is not sufficient data, however, in the literature to determine either seasonal variation, the effect of altitude, or the various climatic conditions, to enable us to discuss our present results in relation to these observations in man. Neither are we prepared to discuss the underlying mechanism which brings about these remarkable changes in the blood picture from a single exposure to dry heat, nor will we attempt to offer an explanation for this at the present time. For purposes of further investigation it offers a method by which we can produce a marked and durable increase in the circulating lymphocytes, thus affording a further opportunity for the study of the function of these cells.

The second point of interest is the large number of lymphocytes found in the circulation in the process of what appears to be an amitotic division. The majority of biologists consider that amitotic division is a degenerative process, and they are inclined to cast doubt on the possibility of the development into normal functioning cells. In either case we know that after heating there is a large increase in what appear to be normal circulating lymphocytes of both the large and small type. At present it is impossible to say positively where they have arisen. It seems probable that there is a sufficient stimulation of the lymphoid centers to account for the increase.[15]

## SUMMARY.

Animals subjected to dry heat for a short period show a sharp fall in the total white blood count, both the polymorphonuclear leucocytes and the lymphocytes taking part in the fall. Following this there is a slow recovery on the part of the polymorphonuclear leucocytes, which generally require several weeks to regain their normal number. The lymphocytes rise rapidly after the initial fall and continue to rise for 2 or 3 weeks. This increase often amounts to a gain of over 200 to 300 per cent above the normal count for the animal. The observations were made on mice, rats, and guinea pigs.

The circulating lymphocytes during the more active stage of stimulation after heating show numerous examples of amitotic division.

[15] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17.

## EXPLANATION OF PLATES.

### Plate 1.

Fig. 1. Microphotograph from a blood film of a mouse 6 days after an exposure to heat, showing various stages of amitosis in the lymphocytes.

### Plate 2.

Fig. 2. Drawing of various stages of amitosis seen in the lymphocytes of the blood of a mouse 8 days after exposure to heat.

### Plate 3.

Fig. 3. A single microscopic field from a blood film of a mouse 8 days after exposure to heat, showing four irregular lymphocytes.

# THE SOURCE OF THE LYMPHOCYTOSIS INDUCED BY MEANS OF HEAT.

By WARO NAKAHARA, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 4 to 7.

(Received for publication, October 17, 1918.)

A recent article from this laboratory reported experiments showing the effect of dry heat on the blood picture in mice.[1] After a 5 minute exposure of the animals to heat ranging from 55–65°C. there was an immediate fall in the circulating white cells. This was followed by a sharp rise in the lymphocytes which increased to a point about 200 per cent above their initial number. The increase extended over from 2 to 3 weeks and later gradually subsided to the normal level. The polymorphonuclear leucocytes participated in the initial fall but recovered very slowly and rarely went above the normal number. The lymphocytes resulting from this stimulation appeared to be normal, healthy cells of both the large and small type. It was noted that during the most active proliferative stage a number of lymphocytes were in the process of typical amitotic division. It seemed doubtful, however, in the light of the almost generally accepted opinion of biologists that this process ever gives rise to normally functioning cells. As the heat stimulation produces normal looking cells, it seemed desirable to study the other possible sources of origin.

## *Source and Type of Material.*

The material for this study was collected by Murphy and Sturm[1] from a number of mice carried as a parallel to an experiment in which blood studies were made. These mice were of the same stock and about the same age. They were subjected to an exposure of dry heat for 5 minutes at a temperature ranging from 55–65° C. Several

[1] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.

of these mice were killed immediately after the heat exposure and the others in groups at intervals of 2, 4, 6, 8, 12, and 14 days. The spleen, lymph glands, and other organs were fixed in Flemming's, Bouin's, or Zenker's fluid. Sections were cut from 5 to 12 microns thick, and most of them stained with Heidenhain's iron-hematoxylin, eosin-methylene blue, and a few with Ehrlich's triacid stain. Of this material, the spleen and lymph glands have been studied and the results will be briefly described.

## OBSERVATIONS.

*Spleen.*—The spleen becomes decidedly changed immediately after the treatment with heat. An examination reveals that there are a large number of cells degenerated or tending to degenerate (Fig. 1). Pyknosis is present not only in the cells of the nodules, but also in the pulp. The cell mass of the germinal center, however, seems always to remain unaffected (Fig. 2). There is no evidence of proliferative activity in the germinal center in the nodule, and only rarely can a mitotic figure be seen. There is also an apparent decrease in the number of the megalokaryocytes.

Sections taken 48 hours after the treatment present an appearance similar to that seen in the normal animals, the differences being an excessive number of necrotic cells, a relative scarcity of megalokaryocytes, and a great number of mitotic figures in the germinal center in the nodules. The active proliferation of cells in the germinal center (Fig. 3) is of significance in view of the fact that in the normal spleen of the adult the cells in the germinal center are not usually actively proliferating, while in infancy, when the lymphoid elements are actively formed, mitosis is of usual occurrence. These facts suggest that the occurrence of the mitotic figures in the germinal center, following the widespread degeneration, is primarily for the purpose of restoring the normal condition in the organ. The phenomenon may thus be regarded as one of overregeneration.

Sections taken 4 days after the treatment show that the spleen is well on the way to recovery (Fig. 4). Here necrotic cells are much less abundant, although they persist, especially in the pulp and in the periphery of the nodule. As in the normal spleen, the association

of mitotic figures with the germinal center is inconstant. The megalokaryocytes, which had been more or less decreased, are now present in fairly large numbers.

A condition similar to that last described is shown in all the sections taken later, after the treatment. That the megalokaryocytes are more, and necrotic cells less abundant in sections taken later may be looked upon as indicating complete recovery.

Macroscopically, the organ apparently undergoes marked enlargement at about the 6th to 8th day after the treatment. I have observed on the 8th day a spleen approximately four times the normal size. At the 14th day the size of the organ is seen to be normal. The size of the spleen varies greatly, even among apparently normal mice, but the fact of its enlargement after the treatment seems, in a general sense, to be indisputable.

*Mesenteric Lymph Gland.*—The cortex of the mesenteric lymph gland becomes full of necrotic cells immediately after the treatment (Fig. 5). The central cell mass of the nodules (germinal center) is apparently normal, but as a rule, no mitotic figures are seen in it. Numerous necrotic cells are observed in lymph cords, and no mitotic figures are seen. The pulp spaces are apparently normal.

After 48 hours the nodules are almost free of necrotic cells, although these cells are present in other parts of the cortex. Cells in the germinal center are actively proliferating, as evidenced by large numbers of mitotic figures in that region (Fig. 6). In lymph cords numerous necrotic cells and few mitotic figures are seen. The appearance of large multinucleated cells, probably phagocytic, is noted in the pulp spaces.

Conditions after the 4th up to the 14th day are apparently normal (Fig. 7). Mitosis in the germinal center is also of normal frequency, judging from the number encountered in sections.

Grossly, there is a general enlargement of the gland at about the 8th day after the treatment. The lymph glands taken at 14 days are approximately of normal size.

*Inguinal Lymph Gland.*—The changes observed here are exactly similar to those found in the mesenteric gland. Numbers of necrotic cells (Fig. 8) and suppression of the proliferative activity in the germinal centers are the fundamental characteristics immediately

after the treatment. After 48 hours necrotic cells become almost entirely eliminated from the cortex, and the germinal center assumes an appearance of great proliferative activity (Fig. 9). In the medulla there are still a number of necrotic cells persisting, but isolated cases of mitosis in lymph cords are not infrequent.

Histologically, the gland after the 4th day of the treatment is apparently normal. Here, also, as in the mesenteric gland, there is an apparent abnormal enlargement of the gland at about the 8th to 10th day after the treatment.

## DISCUSSION.

One of the most striking findings in the observations described above is the complete parallel in the changes undergone by the splenic cells and the cells of the lymph glands. The first change observed in the spleen and in the two kinds of lymph glands, after the treatment with heat, is the widespread degeneration in their cell elements. Within 48 hours, or perhaps much earlier, the germinal centers become active, as demonstrated by the abundant mitotic figures in them, and they thus function in the restoration of the cellular elements of the organs. The germinal centers, however, later become inactive again.

In all the cases, both in the spleen and in the lymph glands, there is a marked increase of mitotic figures in the germinal center, following the general necrosis. The frequency of mitosis in the region apparently exceeds by far that in the normal animal, and it is natural to suppose that the enhanced cell proliferation accounts for the overproduction of lymphocytes. That the cell multiplication here is more than compensation for the degenerated cells is evident in light of the fact that dimensional enlargement of the organ, often well marked, follows the activity of the germinal center. The enlarged spleen and lymph glands are filled with normal cells, and it seems to be indisputable that excessive proliferation of the cells is responsible for their altered appearance. An attempt was made to ascertain whether there is definite increase in number and expansion in the area of germinal centers, after they are stimulated, but it has not been successful because of the unfavorable nature of the structure for such study.

It has been emphasized that a large number of cells in the spleen and in the lymph glands degenerate from the effect of heat immediately after its application. If, then, the cells in the spleen and lymph glands are a source of lymphocytes, there should be a fall in the number of these cells in the circulating blood following the application of heat. Murphy and Sturm have shown this to be the case. This corollary, taken together with other better known facts, tends to warrant the conclusion that the source of the induced lymphocytosis is at least partly the lymphoid cells in the spleen and lymph glands.

Results of recent experiments carried on in this laboratory point to the fact that a certain dose of x-rays can be used to produce a lymphocytosis similar to that brought about by heat.[2,3] The results of blood counts after suitable treatment of the animal with this agent prove that the lymphocytic change is parallel to that in the case of heat treatment, with the characteristic fall in lymphocyte count preceding the marked rise.

Attention may be called to the results of Heineke[4] and Warthin,[5] who have shown that the effect of x-rays on lymphoid tissues is, in the main, similar to that of heat, as described in the present paper. Their data, however, do not throw any light upon the question as to whether there is any proliferation of lymphoid cells above the normal in the process of regeneration, as they did not make observations during the critical period after the x-ray treatment, when an excessive multiplication of the cells may possibly take place. For its satisfactory solution, therefore, the problem must be reinvestigated from a standpoint different from that of the earlier workers.

The induced lymphocytosis is not due to the direct action of heat on lymphoid cells, but is due to proliferative activity on the part of the cells. In other words, heat is primarily a destructive agent of the lymphoid cells, but it causes secondary multiplication of the cells, thus bringing about pronounced lymphocytosis.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[3] Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.

[4] Heineke, H., *Mitteil. Grenzgeb. Med. u. Chir.*, 1905, xiv, 21.

[5] Warthin, A. S., *Physician and Surg.*, 1907, xxix, 1.

## SUMMARY AND CONCLUSION.

A striking number of mitotic figures have been observed in the germinal center of the spleen and lymph glands during the regeneration of the cellular elements of these organs after the destructive effect of heat. This enhanced cell proliferation is interpreted as more than compensating for the degenerated cells, because of the subsequent enlargement of the organs. It has also been pointed out that the characteristic decrease in the number of lymphocytes immediately after the heat treatment is always accompanied by an extensive cell degeneration in spleen and lymph glands at the corresponding period.

On this basis it seems evident that the pronounced lymphocytosis induced by means of heat treatment of the animal is due, at least in part, to the enhanced proliferative activity of germinal centers in the spleen and lymph glands, reacting to the destructive effect of heat upon lymphoid cells.

## EXPLANATION OF PLATES.

### PLATE 4.

FIG. 1. A part of the spleen, immediately after the treatment, showing necrotic cells around the inactive germinal center. × 450.

FIG. 2. The same, showing normal cells of the germinal center (above), and necrotic cells around it (below). × 1,000.

### PLATE 5.

FIG. 3. Germinal center of the spleen, 48 hours after the treatment. *M*, mitotic figures. × 1,000.

FIG. 4. Germinal center of the spleen, 4 days after the treatment, showing the condition to be apparently normal. × 1,000.

### PLATE 6.

FIG. 5. Part of cortex of the mesenteric lymph gland, immediately after the treatment, showing marked necrosis. × 1,000.

FIG. 6. Germinal center of the mesenteric lymph gland 48 hours after the treatment. *M*, mitotic figures. × 1,000.

FIG. 7. The mesenteric lymph gland 12 days after the treatment, showing the germinal center (left) and cells around it. × 1,000.

## Plate 7.

Fig. 8. Part of the medulla of the inguinal lymph gland, immediately after the treatment, showing necrotic cells. × 1,000.

Fig. 9. Germinal center of the inguinal lymph gland 48 hours after the treatment. *M*, mitotic figures. × 1,000.

# THE LYMPHOCYTES IN NATURAL AND INDUCED RESISTANCE TO TRANSPLANTED CANCER.

## IV. Effect of Dry Heat on Resistance to Transplanted Cancer in Mice.*

By JAMES B. MURPHY, M.D., and ERNEST STURM.

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(Received for publication, October 18, 1918.)

The striking histological difference between a cancer graft in an immune animal and in a susceptible animal is the early appearance of large numbers of lymphocytes in the former and the relative absence of these cells in the latter. This fact has led some observers to venture the opinion that the lymphocytes may be a factor in the resistance to these growths. Da Fano[1] carried out extensive histological studies of this reaction about the cancer graft in immune animals and also extended his observations to changes in the cells of the subcutaneous tissue of the body. He demonstrated a marked increase in the lymphoid elements about the cancer graft and in the tissues, and likewise showed an increase in the closely related group of plasma cells. Later Murphy and Morton[2] showed that mice potentially immune to cancer developed a marked lymphocytosis after inoculation with a cancer graft. These investigators also showed that potentially immune animals can be rendered susceptible to cancer inoculation if the lymphoid tissue is depleted by means of x-rays. Murphy and Taylor[3] extended the latter observation and showed that immune animals of a tested resistance can be made again susceptible to inoculation after depletion of the lymphoid tissue.

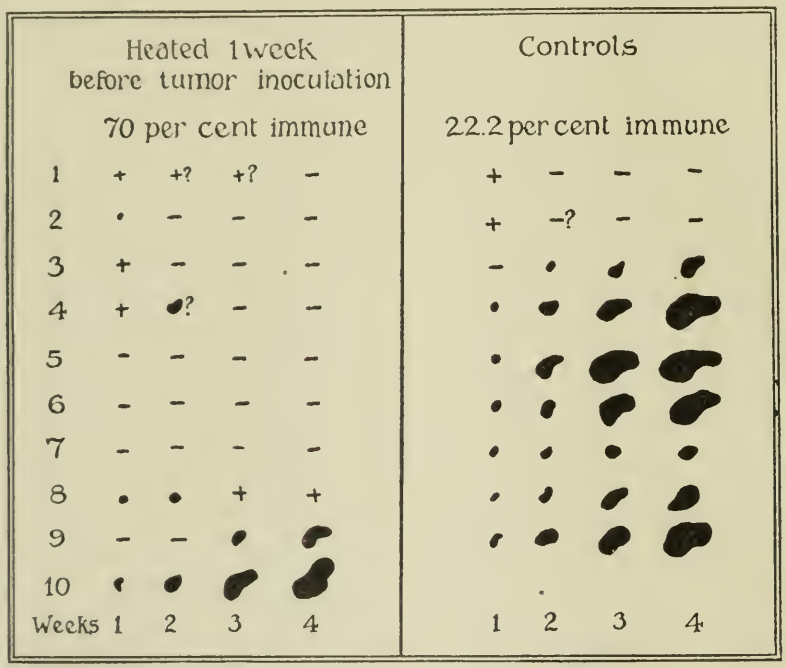
*This investigation was carried out by means of funds from the Rutherford Donation.

[1] Da Fano, C., *Z. Immunitätsforsch., Orig.*, 1910, v, 1.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[3] Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1.

It has been deemed advisable to test the effect of induced lymphocytosis in the animal on resistance to inoculated cancer. The amount of stimulation of the lymphocytes induced by splenectomy[4] and small doses of x-rays[5] did not prove sufficient to influence markedly the course of these highly resistant transplantable tumors. However, with the development of the more extensive and enduring stimulation induced by heat we have an opportunity of testing the effect of such a reaction on cancer resistance.



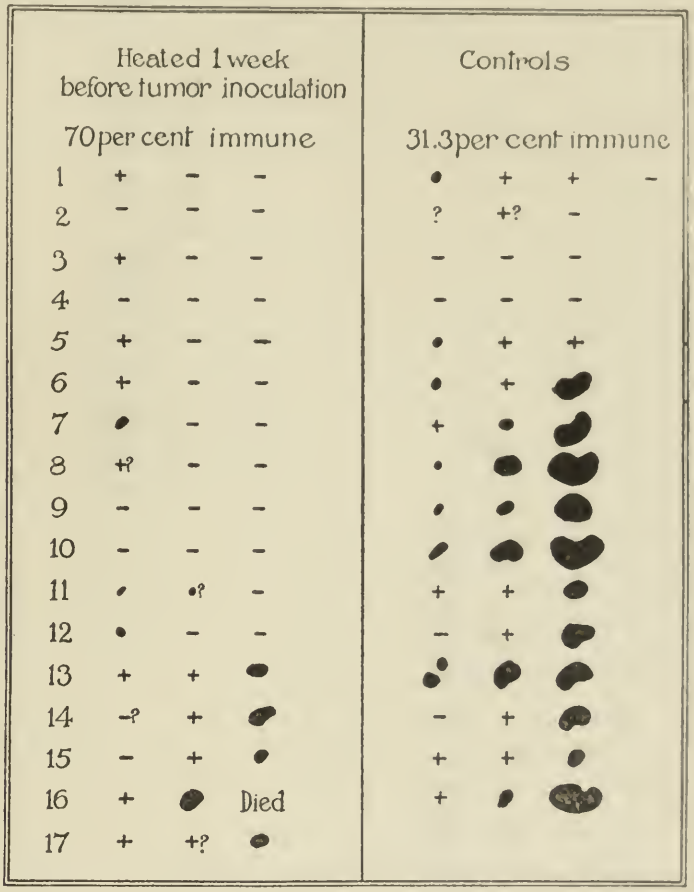

TEXT-FIG. 1. Rate of growth of the Bashford Adenocarcinoma No. 63 in mice heated 1 week before inoculation contrasted with the rate of growth in control animals.

*Experiment 1.*—Ten mice of about the same age and weight were heated for 5 minutes at 55–65°C. over an electric heat lamp. 1 week later the animals, together with ten healthy controls, were inoculated subcutaneously in the groin with a strain of a 3 week old transplantable cancer (Bashford Adenocarcinoma No. 63). The rate of growth of the tumors was charted at weekly intervals thereafter. At the end of 4 weeks the heated animals showed only three tumors, with an immunity of 70 per cent. Of the nine control animals surviving, seven developed tumors, and only two were immune. This per cent of immunity (22.2)

[4] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.
[5] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

is in striking contrast to the 70 per cent in the heated animals. The results of this experiment are shown in Text-fig. 1.

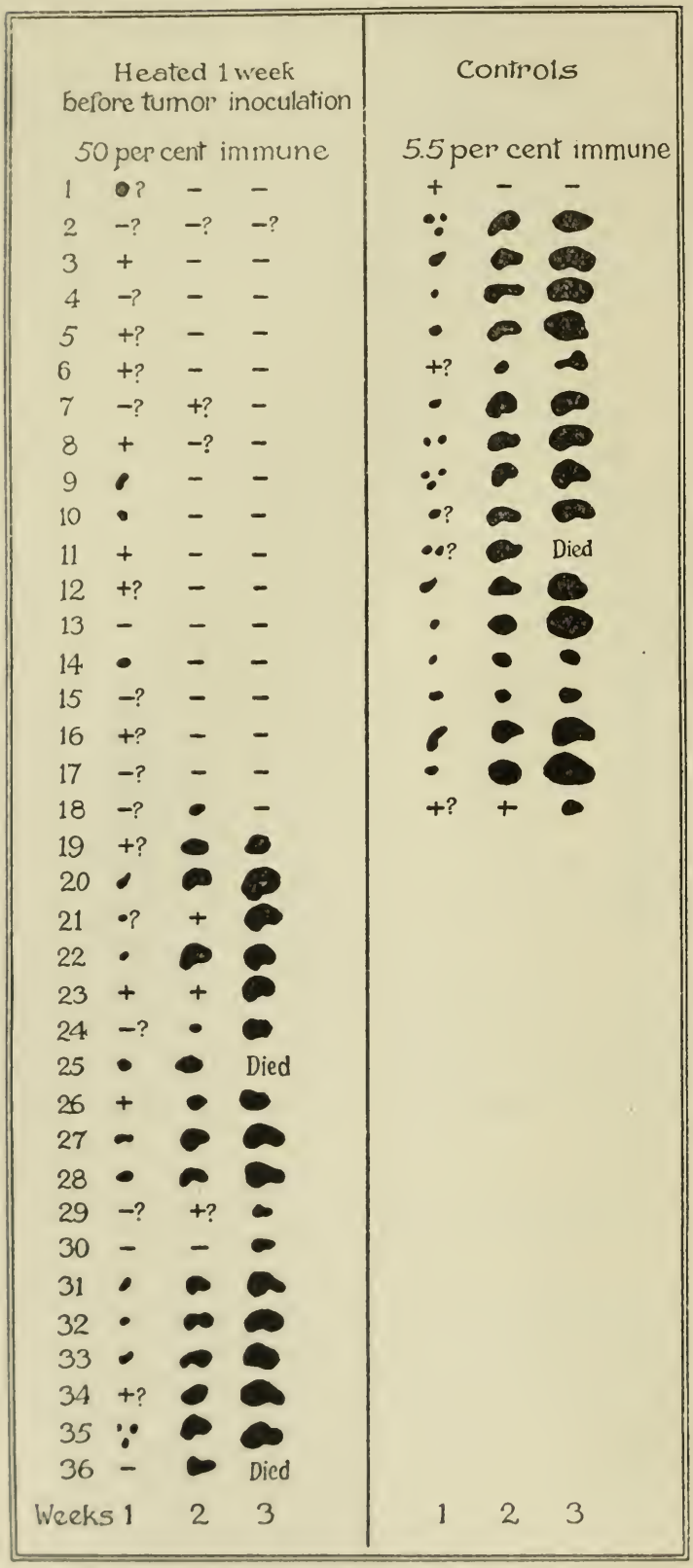
*Experiment 2.*—Seventeen mice were heated in the manner described in the previous paragraph. A week later these animals, with sixteen control mice, were inoculated with a strain of the Bashford Adenocarcinoma No. 63. Weekly measurements were made of the developing tumors. The heated animals showed five out of seventeen with tumors, or an immunity of 70 per cent. Eleven of the sixteen control animals developed tumors, the immunity being 31.3 per cent.




TEXT-FIG. 2. The same as Text-fig. 1.

The difference in immunity between the heated animals and the controls presents a striking contrast. The result is shown in Text-fig. 2.

*Experiment 3.*—Thirty-six healthy mice of about the same age and size and from the same stock were heated for 5 minutes over an electric heat lamp at a temperature ranging from 55–65°C. A week later these animals, with eighteen normal mice, were inoculated with a 2½ week old Bashford Adenocarcinoma No. 63. After 3 weeks eighteen of the thirty-six heated animals had developed tumors, showing an immunity of 50 per cent, while seventeen of the eighteen con-




TEXT-FIG. 3. The same as Text-fig. 1.

trols had developed tumors, the immunity being only 5.5 per cent. This experiment is shown graphically in Text-fig. 3.

The three foregoing experiments, carried out on over 100 mice, show that animals whose lymphocytes have been stimulated by dry heat have a much higher resistance to transplanted cancer than control mice inoculated with the same tumor. The general health of animals subjected to this treatment did not seem in the least affected. There was no loss of weight, no roughening of the hair, or other indication of disturbance. This difference in resistance was manifest both when the tumor inoculated gave a relatively low per cent of takes, and when the tumor was highly virulent and gave a high per cent of takes.

## DISCUSSION.

The absence of any acceptable demonstration of antibodies to explain cancer immunity suggests strongly that this type of resistance probably comes under the head of cellular immunity. The evidence connecting the lymphocyte with the resistance to transplantable cancer may be summed up briefly as follows: (*a*) the presence of lymphocytes and related cells about a cancer graft in immune animals, and the relative absence of these cells around such a graft in highly susceptible animals; (*b*) the general changes which take place in the cellular elements of the tissues of animals potentially immune to cancer (Da Fano); (*c*) the lymphocytic crisis in the circulating blood of potentially immune animals after inoculation with cancer; (*d*) destruction of potential cancer immunity by depletion of the lymphoid elements with x-rays; (*e*) destruction of established cancer immunity by the same means; (*f*) the marked increased resistance to cancer after artificial stimulation of the lymphocytes.

It would be difficult to adapt the foregoing facts so that they would fit either into the Ehrlich theory of cancer immunity or that of the Bashford school of stroma reaction. Neither of these explanations seems tenable in the light of the present results. That other factors than the lymphocytes are involved in the process of cancer immunity seems more than probable. It can at least be said with a degree of certainty that we have in the lymphoid elements an important link in the process of so called cancer immunity.

## SUMMARY.

The marked and durable stimulation of the lymphoid elements induced by dry heat applied to the animal results in the establishment of a high degree of immunity to certain transplantable cancers in mice. This immunity is evident when the tumor used gives a low, as well as when it gives a high percentage of takes.

# EFFECT OF STIMULATION OF THE LYMPHOCYTES ON THE RATE OF GROWTH OF SPONTANEOUS TUMORS IN MICE.*

By JAMES B. MURPHY, M.D., and ERNEST STURM.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 21, 1918.)

Murphy and Morton[1] have shown that mice with spontaneous tumors when exposed to small doses of x-rays sufficient to stimulate somewhat the lymphocytes exhibit an increased resistance to replants of their own tumors. In these experiments the tumors were removed by operation and with the tumor out the animal was exposed to a suitable small dose of x-rays. Immediately afterwards a graft of the original tumor was reinoculated into the groin of the mouse. Another series of mice with spontaneous tumors from the same strain was submitted to the same procedure, except that the x-ray treatment was omitted. 50 per cent of the x-rayed mice showed no growth of the returned graft or local recurrence of the cancer. The other 50 per cent showed a growth of the graft which appeared at a much later period than in the untreated animals. In the control group of animals, in which the x-ray treatment was omitted, over 96 per cent of the animals showed a growth of the returned graft. The latter figure corresponds to that obtained by other observers with similar experiments.

In the work to be reported here we have used dry heat as the method of stimulation instead of x-rays. A mouse exposed for 5 minutes to dry heat at a temperature ranging from 55–63°C. will show at first a slight fall in the circulating lymphocytes, followed by a marked increase, lasting from 2 to 4 weeks.[2] Accompanying the increase in

* This investigation was carried out by means of funds from the Rutherford Donation.

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[2] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix 1.

the circulating lymphocytes there is a marked activity on the part of the lymphoid organs evidenced by numerous mitotic figures in the germinal centers.[3]

*Experiment.*—The animals for this experiment were procured from the Lathrop stock.[4] There were 61 mice showing numerous gradations and types of mammary carcinomas. The tumors were removed as completely as possible by operation, and while the tumor was out the animal was heated for 5 minutes over an electric heat lamp at a temperature of 55–63°C. Immediately after heating, a graft of the original tumor was reinoculated subcutaneously into the left groin of the animal. In 36 of the 61 animals so treated a complete immunity to recurrence of the growth of the graft resulted. There were no metastases observed in any of these 36 animals. Only those mice were included in the series which lived for a period of over 4 weeks and remained in good condition. The majority lived for a much longer period than this. Among the 25 animals of this series which were not immune, the average time for the graft to become large enough to be recognized was 2 weeks and 5 days. The number of recurrences was seven. In some instances there was a recurrence which later retrogressed, but these have not been recorded as immune animals.

For controls we used our former series of animals which were taken from the same stock and were treated in the same way except that they were not heated. Of twenty-nine animals the returned graft grew in twenty-eight, and the average time required for the graft to become palpable was 1 week and 5 days. Local recurrences were present in fourteen out of the twenty-nine animals. Table I gives a comparison of the two series.

TABLE I.

| Series No.* | Immune. | Susceptible. | Local recurrences. | Grafts alone. | Questionable grafts. | Metastasis alone. | Time for appearance of graft. |
|---|---|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| I | 59.4 | 40.6 | 11.3 | 14.7 | 8.1 | 6.5 | 2 wks. and 5 days. |
| II | 3.4 | 96.6 | 48.3 | 48.3 | | | 1 wk. " 5 " |

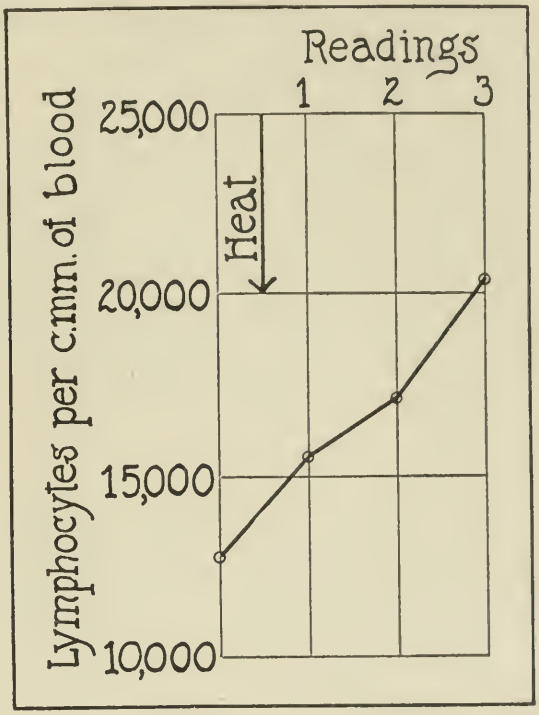
* Series I. 61 mice with spontaneous cancers heated after the removal of the tumor with later a return of a graft.

Series II. 29 control mice with cancer removed at operation and later a return of a graft.

[3] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17.

[4] The Lathrop stock of mice was recently purchased for The Rockefeller Institute for Medical Research by funds from the Rutherford Donation.

Blood counts were made on about half of the animals of the treated series, one before operation, and the next 1 week after operation and treatment, and subsequent counts were made at weekly intervals (Text-fig. 1). The average number of lymphocytes per c.mm. of blood before operation was about 12,000. 1 week after operation and heating they had risen to approximately 16,000, and they continued to increase until by the fourth count there were 20,000 lymphocytes per c.mm. of blood. In twenty of thirty-eight animals counted the polymorphonuclear leucocytes were lower after treatment than before. In the other eighteen, except in two or three cases where extensive infection occurred, there




TEXT-FIG. 1. The average number of lymphocytes of mice with spontaneous tumor after having been subjected to an exposure of heat. The counts were made before and at weekly intervals after heating.

was only a slight gain. These figures correspond with those reported for normal animals after exposure to heat. Counts made on untreated animals subjected to the same surgical procedure showed no such change.

## DISCUSSION.

Pathologists from an early date have noted that lymphocytes accumulate about the slowly growing cancers, while they are absent in the more rapidly growing malignant types. This point has received a certain amount of comment but slight attempt has been

made at explanation. Our present results suggest that this infiltration may be of more importance than it has been previously considered. The great obstacle to overcome in accepting the lymphocyte as a factor of resistance to cancer growth is the fact that the lymph glands are the common point of metastases. This fact needs further study and elucidation but is not to be looked upon as an insurmountable barrier. Such possible questions present themselves as: What is the condition of the glands to which metastases take place? Is the resistance offered overcome by the number of cancer cells lodging in the lymph spaces? Are the lymphocytes in the gland in an actively functioning stage or do they require an activating substance like the opsonins? It is impossible to discuss these points with our present knowledge. The experiments reported here, coupled with the previous ones with x-rays, suggest strongly that the lymphoid tissue does offer a resisting influence to cancer growth, but this conception cannot be accepted unreservedly until further light is thrown on the activities of the lymph glands.

## SUMMARY.

Spontaneous cancers were removed from a series of mice by operation. The animals were then subjected to an exposure to dry heat at a temperature ranging from 55–63°C. for 5 minutes. Immediately afterwards a graft of the original tumor was returned. The mice so treated exhibited a marked increase in their resistance to the growth of the cancer graft, over 59 per cent remaining entirely free from a return of the cancer. In a control series in which no treatment was given 96 per cent of the animals showed a return of the cancer.

# EXPERIMENTS ON THE RÔLE OF LYMPHOID TISSUE IN THE RESISTANCE TO EXPERIMENTAL TUBERCULOSIS IN MICE.

## III. Effect of Heat on Resistance to Tuberculosis.

By JAMES B. MURPHY, M.D., and ERNEST STURM.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 19, 1918.)

In a previous report from this laboratory a series of experiments was recorded which seemed to explain the results of Lewis and Margot,[1] who noted that splenectomized mice had a greater resistance to tuberculosis than normal mice. Murphy and Ellis[2] demonstrated the fact that splenectomized mice, if exposed to suitable doses of x-rays no longer had an increased resistance, but were hypersusceptible to the infection. The interpretation of these results suggested, was that the increased resistance in splenectomized mice was due to the increase in the circulating lymphocytes, which was demonstrated to reach its height about 21 days after removal of the spleen. When the increase in the lymphocytes was prevented by x-rays, the increased resistance to tuberculosis was nullified. It was likewise shown that intact animals could be rendered less resistant to tuberculosis than controls by the use of broken doses of x-rays. The latter observation was confirmed by Morton[3] for the human strain of the organism with guinea pigs. Later Taylor and Murphy[4] showed that mice with a marked increase in the lymphocytes resulting from cancer inoculation in cancer-immune animals had also a marked increase in their resistance to tuberculosis. In this instance also if the increase in lymphocytes was de-

[1] Lewis, P. A., and Margot, A. G., *J. Exp. Med.*, 1914, xix, 187.

[2] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.

[3] Morton, J. J., *J. Exp. Med.*, 1916, xxiv, 419.

[4] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.

stroyed by x-rays, the animals were rendered highly susceptible to infection.

We regarded these results, coupled with the deductions from observations of the blood count in men with this disease, and the histology of the lesion, as strong direct evidence that the lymphocytes play an important part in the resistance of the animal to tuberculosis. With the development of a new method of stimulating the lymphocytes,[5] namely that of intense dry heat, we have another opportunity to test this conception.

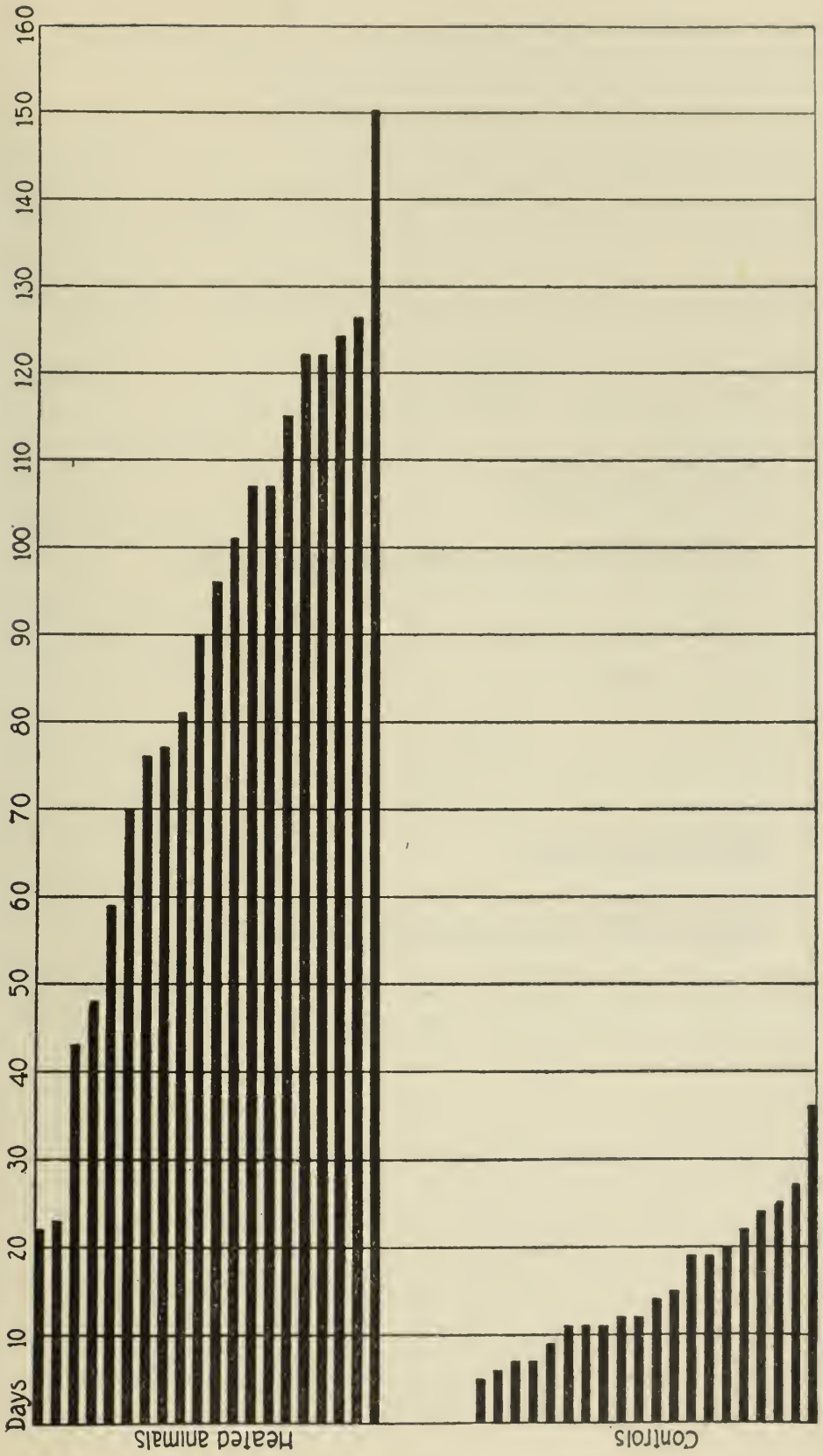
*Method.*

Mice subjected to dry heat ranging from 55–65°C. over a period of 5 minutes first show a fall in the total white blood count, both the polymorphonuclear cells and, to a lesser extent, the lymphocytes participating in the fall. The lymphocytes, however, show an immediate rebound, followed by a rapid increase which carries the count several hundred per cent above the normal. This rise continues for 14 to 21 days, after which there is a gradual return to the normal level. The polymorphonuclear leucocytes recover slowly and show no stimulation phase. This method gives us an excellent opportunity to test the effect of a marked increase in the lymphocytes on the course of tuberculous infections.

*Experiment 1.*—Forty mice of the same stock and of about the same age and size were selected for this experiment. Twenty were heated over an electric heat lamp for 5 minutes at a temperature starting at 55°C. and allowed to increase to 65°C. 1 week later these animals, together with the twenty controls, were each inoculated intraperitoneally with 2 mg. of a bovine strain of tubercle bacilli (4 week culture) suspended in 0.5 cc. of normal salt solution. The mice were then placed in individual jars in order to prevent the occurrence or spread of an epidemic. All the mice as they died were carefully autopsied, and films were taken from the peritoneal fluid, and from liver, kidney, lungs, and heart's blood. It was found that all the animals had widely disseminated tuberculosis, which could definitely be accepted as the cause of death.

The control animals died rapidly, the first one on the 6th day, and the last on the 36th day after inoculation. The average number of days of life for this group was approximately 16 days. None of the heated animals died until the

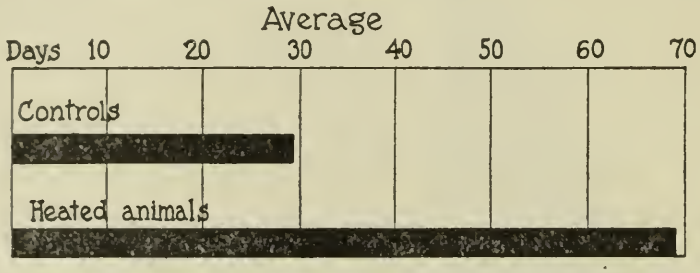
[5] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.



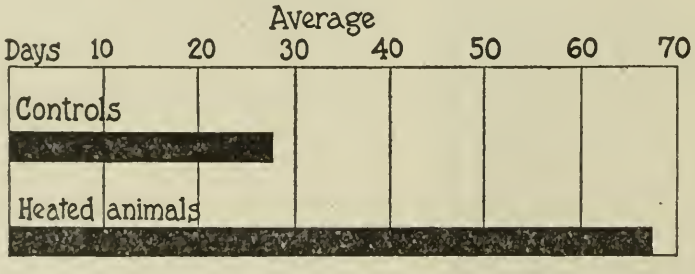

TEXT-FIG. 1. Each horizontal line represents the time of survival of a mouse after inoculation with bovine tubercle bacilli. The first group was subjected to an exposure of dry heat 1 week before inoculation. The second group was untreated.

22nd day after inoculation, and the longest period of life in this group was 150 days, the average length of life for the twenty mice being approximately 88 days. The rate at which the animals died is shown in Text-fig. 1.

*Experiment 2.*—Twelve healthy mice of about the same age and weight were heated in the same manner as in the previous experiment. A week later these, with twelve control mice from the same stock, were inoculated intraperitoneally with 2 mg. of a bovine strain of the tubercle bacillus suspended in 0.5 cc. of normal salt solution. The animals were segregated in individual jars as in the previous experiment. Autopsies were performed promptly after death and films taken from the principal organs, the peritoneal fluid, and the heart's blood.




TEXT-FIG. 2. The average duration of life of heated mice after inoculation with bovine tubercle bacilli compared with the duration of life of untreated animals. There were twelve mice in each group.




TEXT-FIG. 3. The average duration of life of heated mice after inoculation with bovine tubercle bacilli compared with the duration of life of untreated animals. There were twelve heated mice and fourteen controls.

All these mice showed extensive tuberculosis except one control which lived 89 days, and which was either a highly resistant animal or was not infected because of some accident in the inoculation. In this series the heated animals averaged 69 days of life after inoculation, while the controls averaged only 29 days. The details of this experiment are shown in Text-fig. 2.

*Experiment 3.*—Twenty-six mice of the same strain and about the same age were selected for the experiment. Twelve were subjected to direct heat ranging from 55–65°C. for 5 minutes. A week later each of the twenty-six animals was inoculated intraperitoneally with 2 mg. of a culture of the bovine tubercle bacillus suspended in 0.5 cc. of normal salt solution. They were segregated in separate

jars as in preceding experiments. Autopsies were performed as the animals died to verify the cause of death. In all, except one of the control animals, there was widely disseminated tuberculosis. In this animal, which lived 117 days, there was no evidence of the disease.

In this experiment the heated animals averaged 67.6 days of life after inoculation, while the controls lived only 27.8 days. The rate at which the mice died is shown in Text-fig. 3. In the last two experiments the same culture of the tubercle bacillus was used for inoculation.

The three experiments described show that animals subjected to one exposure of dry heat and inoculated a week later with a bovine strain of the tubercle bacillus virulent for mice had a greatly enhanced resistance to the organism as compared with that of the untreated animals. The difference in resistance here is much more striking than that seen in splenectomized animals or in the cancer-immune animals.

## DISCUSSION.

The impression is gathered from the literature that individuals recovering from tuberculosis develop an increase in the number of circulating lymphocytes, although the point is rarely emphasized. In an analysis of a number of blood counts on rapidly advanced cases of tuberculosis in man a marked decrease in this type of cell is observed. The fact that between these two characteristic findings there is every variation in the type and degree of reaction, with often practically normal blood counts, has led to skepticism in regard to the significance of the blood changes in tuberculosis. In dealing with such a slow, chronic infection, the changes would not be expected in a marked degree except in the two extremes mentioned above. The blood changes, together with the fact that the tuberculous lesion is characterized by an accumulation of large numbers of lymphocytes, are suggestive of the part played by this cell in resistance to tuberculosis. To this evidence we add the results of our experiments; namely, the lowering of resistance to tuberculosis in animals depleted of their lymphocytes by means of x-rays, and the increased resistance in animals with the lymphocytes increased by three widely different methods, splenectomy, cancer immunity, and dry heat.

The chief points which may be brought against this conception is that tuberculosis frequently involves the lymphoid structures, and

they seem, if anything, more susceptible to the infection than many other tissues. The explanation of this fact is not clear, but we do not consider it an overwhelming argument in the face of the mass of evidence which indicates that the lymphocyte is an important factor in the resistance to the tubercle bacillus.

## SUMMARY.

Mice with high lymphocyte counts and increased activity of the lymphoid tissue induced by one exposure to intense dry heat exhibit a marked increase in the resistance to large doses of bovine tubercle bacilli as compared with that shown by control animals given a similar inoculation. This resistance, judged by the time of survival after inoculation, is increased from two- to threefold. The average length of life after inoculation for three groups of heated mice was 88, 69, and 67 days respectively, while the control groups averaged 16, 29, and 28 days respectively.

# EFFECT OF EXPOSURE TO THE SUN ON THE CIRCULATING LYMPHOCYTES IN MAN.

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Experiments reported previously[1-4] indicate that in animals a blood lymphocytosis attends increased resistance to cancer and tuberculosis. The work has shown that the number of circulating lymphocytes can be varied by suitable dosage of the Roentgen rays. Massive x-ray exposures decrease, while small ones increase, both proportionately and actually, the number of lymphocytes in the circulating blood. It seemed possible that the beneficial results of heliotherapy in surgical tuberculosis noted by Rollier and others[5] might be due, in part at least, to a similar effect of the actinic rays on the lymphoid organs, leading to an increase in the number of circulating lymphocytes. The fact that heliotherapy is applied chiefly at high altitudes in the Alps suggested also that the actinic rather than the heat rays were the therapeutic agents. The ultraviolet rays contained in light of solar origin lie in that portion of the invisible spectrum included between 4,000 and 2,950 Ångström units.[6] Light waves of lengths included between 2,100 and 2,800 and, to a lesser extent, those up to 4,000 Ångström units are bactericidal.[7] However, as shown by Newcomer as well as by previous observers, this action is lost in the presence of organic matter in minute traces. Inasmuch as the surgical lesions treated successfully in this manner

[1] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[3] Morton, J. J., *J. Exp. Med.*, 1916, xxiv, 419.

[4] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.

[5] For a review of the subject see: Roatta, G. B., L'Elioterapia nella pratica medica e nell 'educazione, Milan, 1914.

[6] Bovie, W. T., *Bot. Gaz.*, 1915, lix, 149.

[7] Newcomer, H. S., *J. Exp. Med.*, 1917, xxvi, 841.

are deep seated and the ultra-violet rays have but feeble penetrating power, it does not seem probable that a direct destructive effect on their bacterial causes is responsible for the clinical improvement reported. Fresh air, good food, and rest, in the absence of sunlight do not produce a corresponding effect.

In order to determine whether exposure to the sun has any effect on the circulating lymphocytes of healthy individuals, the following study was made.

A series of blood counts was made, in the spring of 1916, on several individuals who expected to be exposed to the sun during the ensuing summer months at Woods Hole, Massachusetts. In the fall a second series of counts was made on the same subjects, and this report is based on observations made on the bloods of 38 persons who showed more or less evidence of chronic solar dermatitis (tanning) at the time the final blood count was made. Most of the individuals studied were tanned over a large portion of their bodies, and no instances are included in which there was tanning merely of the face and hands. The subjects included both sexes and ranged in age from 20 to 65 years. It is admitted that tanning of the skin is caused by actinic and not by heat rays.

*Method.*

The blood to be counted, obtained from a needle prick in the tip of the finger, was diluted 1:20 with 3 per cent acetic acid solution, the mixture shaken in a diluting pipette for 5 minutes, a drop placed on a Türck hemocytometer, and an interval allowed for the corpuscles to settle. The white corpuscles in 8 of the 9 ruled millimeter squares of the counting chamber were counted and the number per c.mm. was estimated in the usual way. Films made at the same time on cover-slips were used for differential leucocyte counts. After staining with Wright's stain, 300 cells were counted and the percentage of each type was determined. The total number of lymphocytes per c.mm. of blood was estimated by multiplying the total number of white corpuscles per c.mm. by the percentage as determined by differential count.

The results of the study, for convenience arranged in Tables I to VIII, are analyzed in the discussion which follows.

*Discussion of Tables.*—A study of the tables brings out the fact that the blood of persons in whom chronic solar dermatitis is present shows not infrequently considerable percentage and actual increase of the lymphocytes. Thus in 25, or 65.8 per cent of the 38 persons

TABLE I.

*Individuals Showing an Increase in Lymphocytes after Exposure to Sun.*

| Individual No. | Lymphocytes. | | Increase. |
|---|---|---|---|
| | Before exposure to sun. | After exposure to sun. | |
| | *per cent* | *per cent* | *per cent* |
| 1 | 28.1 | 43.8 | 55.5 |
| 2 | 26.3 | 31.7 | 20.8 |
| 3 | 33.0 | 39.0 | 18.1 |
| 4 | 19.6 | 25.3 | 29.0 |
| 5 | 30.0 | 32.0 | 8.6 |
| 6 | 26.0 | 36.0 | 38.5 |
| 7 | 28.0 | 34.0 | 21.4 |
| 8 | 22.6 | 32.6 | 44.2 |
| 9 | 35.0 | 42.6 | 21.7 |
| 10 | 30.3 | 37.0 | 22.1 |
| 11 | 38.6 | 47.3 | 22.5 |
| 12 | 20.0 | 22.0 | 10.0 |
| 13 | 22.6 | 29.6 | 30.9 |
| 14 | 26.0 | 35.0 | 36.9 |
| 15 | 19.6 | 42.2 | 115.3 |
| 16 | 33.6 | 47.2 | 40.5 |
| 17 | 27.1 | 33.0 | 21.8 |
| 18 | 22.0 | 25.6 | 16.4 |
| 19 | 28.0 | 33.6 | 20.0 |
| 20 | 23.6 | 25.9 | 9.7 |
| 21 | 30.0 | 42.8 | 42.7 |
| 22 | 28.0 | 31.8 | 13.6 |
| 23 | 34.6 | 39.0 | 12.7 |
| 24 | 26.0 | 42.4 | 63.1 |
| 25 | 27.0 | 39.6 | 46.6 |
| Average..... | 27.4 | 35.6 | 27.2 |

studied, there was a definite increase in the number of circulating lymphocytes after development of solar pigmentation which averaged 27.2 per cent. This increase, given in the column headed "Increase" in Table I, represents the percentile rise of lymphocytes above the

TABLE II.

*Individuals Showing a Decrease in Lymphocytes after Exposure to Sun.*

| Individual No. | Lymphocytes. | | Decrease. |
|---|---|---|---|
| | Before exposure to sun. | After exposure to sun. | |
| | *per cent* | *per cent* | *per cent* |
| 26 | 34.6 | 32.2 | 6.9 |
| 27 | 42.3 | 33.0 | 21.3 |
| 28 | 35.5 | 28.0 | 21.1 |
| 29 | 41.3 | 29.1 | 29.5 |
| 30 | 42.0 | 36.3 | 13.6 |
| 31 | 42.3 | 37.6 | 11.1 |
| 32 | 43.8 | 42.7 | 2.5 |
| 33 | 34.6 | 32.6 | 5.8 |
| Average..... | 39.6 | 33.9 | 15.2 |

TABLE III.

*Individuals Showing No Change in Lymphocyte Percentage after Exposure to Sun.*

| Individual No. | Lymphocytes. | | Increase. | Decrease. |
|---|---|---|---|---|
| | Before exposure to sun. | After exposure to sun. | | |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| 34 | 29.3 | 30.0 | 2.4 | |
| 35 | 33.3 | 33.0 | | 0.9 |
| 36 | 18.0 | 17.6 | | 2.2 |
| 37 | 27.5 | 26.9 | | 2.2 |
| 38 | 27.0 | 27.8 | 2.9 | |
| Average..... | 27.0 | 27.0 | 5.3 | 5.3 |

TABLE IV.

*Summary of Bloods Studied.*

| Individuals with. | No. | Per cent. |
|---|---|---|
| Lymphocytes increased after exposure to sun..... | 25 | 65.8 |
| " decreased " " " " ..... | 8 | 21.0 |
| " showing no change after exposure to sun..... | 5 | 13.2 |
| Total..... | 38 | 100.0 |

original percentage of these cells. In eight, or 21 per cent, there was an average decrease in the percentage of lymphocytes amounting to 15.2 per cent (Table II). In five, or 13 per cent, there was no substantial change in the lymphocyte percentage (Table III).

If one disregards for the moment the individuals, 5 in number, whose blood showed no definite percentage change, it follows that of the remaining 33, 25, or 73.8 per cent, exhibit an increase, and 8, or 24.2 per cent, a decrease of the circulating lymphocytes. The changes noted in the number of total lymphocytes before and after tanning tended to parallel the percentage change in the same blood. Thus (Table V) twenty-five bloods which gave a definite percentage lymphocyte increase gave also an average absolute increase from 2,574 to 3,338 cells per c.mm. Moreover, the eight bloods which

TABLE V.

*Average Total Lymphocyte Counts.*

| Individuals (Tables I, II, and III.) | Average before exposure to sun. | Average after exposure to sun. | Increase. | Decrease. |
|---|---|---|---|---|
| | | | *per cent* | *per cent* |
| 25 with percentage increase..... | 2,574 | 3,338 | 29.6 | |
| 8 " " decrease..... | 3,807 | 3,187 | | 16.3 |
| 5 " no change in percentage..... | 2,951 | 3,060 | 3.5 | |

gave a decrease in the percentage of lymphocytes followed the same trend, as the average number of these cells before exposure was 3,807, and after 3,187 per c.mm. It should be noted here that the bloods showing no appreciable change in percentage of lymphocytes gave an average total number per c.mm. which varied only slightly as the result of the exposure, the average before exposure being 2,951 and after exposure 3,060.

The average percentage change in the total lymphocytes per c.mm., after exposure, for each of the three series compares closely with similar determinations of the percentile increase or decrease as given in Tables I, II, and III. The twenty-five persons who showed an average percentile increase of 27.2 showed a total lymphocyte increase of 29.6 per cent. The percentile decrease in the eight individuals included in Table II was 15.2, and the average total lymphocyte

TABLE VI.

*Complete Count on Individuals Showing an Increase in Lymphocytes after Sunburn.*

| Individual No. | White blood corpuscles. | Small lymphocytes. | Large lymphocytes. | Polymorphonuclear neutrophils. | Polymorphonuclear eosinophils. | Polymorphonuclear basophils. | Transitionals and large mononuclears | Lymphocytes. | Absolute lymphocytes. |
|---|---|---|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| 1 | 8,648*† | 24.2 | 3.8 | 66.1 | 3.2 | 0.1 | 2.4 | 28.1 | 2,377 |
| | 6,850 | 40.8 | 3.0 | 48.0 | 3.5 | 0.8 | 3.8 | 43.8 | 2,978 |
| 2 | 7,720 | 20.6 | 5.7 | 65.5 | 1.7 | 1.3 | 5.2 | 26.3 | 2,048 |
| | 8,550 | 27.7 | 3.8 | 60.4 | 3.1 | 0.6 | 3.9 | 31.7 | 2,725 |
| 3 | 9,710 | 28.5 | 4.5 | 59.8 | 2.0 | 0.8 | 4.3 | 33.0 | 3,105 |
| | 6,920 | 34.0 | 5.0 | 58.0 | 0.6 | 0.3 | 2.0 | 39.0 | 2,739 |
| 4 | 13,450 | 15.6 | 4.0 | 76.3 | 0.0 | 0.3 | 3.6 | 19.6 | 2,636 |
| | 10,900 | 23.3 | 2.0 | 67.3 | 3.6 | 0.3 | 3.3 | 25.3 | 2,758 |
| 5 | 7,390 | 26.5 | 3.5 | 65.0 | 1.0 | 3.8 | 0.3 | 30.0 | 2,213 |
| | 8,240 | 27.6 | 5.0 | 65.0 | 0.3 | 0.0 | 1.6 | 32.6 | 2,687 |
| 6 | 15,900 | 22.3 | 3.6 | 69.3 | 2.3 | 0.0 | 2.3 | 26.0 | 4,134 |
| | 7,159 | 32.8 | 3.5 | 53.7 | 1.7 | 0.3 | 8.0 | 36.0 | 2,675 |
| 7 | 9,650 | 25.3 | 2.6 | 62.0 | 3.0 | 0.0 | 7.0 | 28.0 | 2,702 |
| | 7,100 | 29.3 | 4.7 | 57.5 | 4.2 | 0.2 | 4.2 | 34.0 | 2,419 |
| 8 | 10,750 | 20.0 | 2.6 | 75.3 | 0.0 | 0.3 | 1.6 | 22.6 | 2,430 |
| | 9,750 | 31.0 | 1.6 | 63.0 | 1.6 | 0.3 | 2.3 | 32.6 | 3,180 |
| 9 | 7,250 | 31.3 | 3.6 | 56.0 | 4.0 | 0.6 | 4.3 | 35.0 | 2,538 |
| | 5,050 | 40.0 | 2.6 | 46.0 | 4.6 | 0.0 | 6.6 | 42.6 | 2,151 |
| 10 | 14,750 | 26.6 | 3.6 | 57.3 | 8.3 | 1.3 | 2.6 | 30.3 | 4,469 |
| | 8,875 | 34.3 | 2.7 | 53.8 | 4.8 | 0.8 | 3.5 | 37.0 | 3,330 |
| 11 | 8,250 | 37.0 | 1.6 | 55.6 | 2.6 | 0.0 | 3.0 | 38.6 | 3,185 |
| | 11,750 | 43.3 | 4.0 | 46.6 | 1.3 | 0.0 | 4.6 | 47.3 | 5,558 |
| 12 | 9,700 | 16.6 | 3.3 | 77.3 | 0.3 | 1.3 | 1.0 | 20.0 | 1,940 |
| | 11,850 | 21.3 | 0.6 | 68.6 | 5.0 | 0.3 | 4.0 | 22.0 | 2,607 |

*Average counts are given in Tables VI to VIII because in many instances from two to six counts were made before, and a similar number after exposure to the sun.

†The upper row of figures for each individual represents counts before, and the lower row counts after exposure to the sun.

TABLE VI—*Concluded.*

| Individual No. | White blood corpuscles. | Small lymphocytes. | Large lymphocytes. | Polymorphonuclear neutrophils. | Polymorphonuclear eosinophils. | Polymorphonuclear basophils. | Transitionals and large mononuclears. | Lymphocytes. | Absolute lymphocytes. |
|---|---|---|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| 13 | 10,667 | 20.6 | 2.0 | 67.0 | 9.3 | 0.3 | 0.6 | 22.6 | 2,410 |
| | 14,500 | 25.3 | 4.3 | 59.3 | 5.3 | 0.3 | 5.3 | 29.6 | 4,292 |
| 14 | 7,800 | 23.0 | 3.0 | 59.0 | 1.6 | 1.0 | 2.3 | 26.0 | 2,028 |
| | 9,500 | 33.3 | 2.3 | 61.0 | 2.3 | 0.0 | 1.0 | 35.6 | 3,382 |
| 15 | 7,050 | 16.3 | 3.3 | 74.6 | 0.0 | 0.3 | 5.3 | 19.6 | 1,382 |
| | 6,200 | 39.6 | 2.6 | 52.0 | 0.0 | 0.6 | 5.0 | 42.2 | 2,616 |
| 16 | 6,000 | 29.0 | 4.6 | 58.6 | 3.0 | 1.0 | 3.6 | 33.6 | 2,016 |
| | 5,800 | 42.6 | 4.6 | 46.0 | 1.0 | 1.6 | 4.0 | 47.2 | 2,738 |
| 17 | 7,925 | 24.6 | 2.5 | 68.3 | 0.8 | 0.2 | 3.5 | 27.1 | 2,189 |
| | 9,600 | 30.3 | 2.6 | 60.3 | 2.0 | 0.3 | 4.3 | 33.0 | 3,168 |
| 18 | 6,700 | 17.5 | 4.5 | 70.0 | 4.0 | 0.0 | 4.0 | 22.0 | 1,474 |
| | 7,350 | 20.6 | 5.0 | 64.6 | 2.6 | 0.0 | 7.0 | 25.6 | 1,882 |
| 19 | 9,300 | 25.6 | 2.3 | 59.3 | 10.0 | 0.6 | 2.0 | 28.0 | 2,604 |
| | 11,100 | 32.6 | 1.0 | 59.6 | 3.3 | 0.3 | 3.0 | 33.6 | 3,696 |
| 20 | 6,850 | 21.0 | 2.6 | 72.0 | 0.6 | 0.3 | 3.3 | 23.6 | 1,617 |
| | 7,825 | 22.8 | 3.1 | 68.3 | 2.0 | 0.2 | 3.5 | 25.9 | 1,983 |
| 21 | 10,850 | 26.3 | 3.6 | 62.6 | 3.3 | 0.6 | 3.3 | 30.0 | 3,255 |
| | 9,550 | 40.3 | 2.5 | 47.3 | 6.0 | 0.5 | 3.3 | 42.8 | 3,886 |
| 22 | 9,100 | 25.6 | 2.3 | 65.6 | 2.3 | 0.0 | 4.0 | 28.0 | 2,548 |
| | 9,900 | 30.6 | 1.1 | 62.8 | 2.3 | 0.2 | 2.8 | 31.8 | 3,084 |
| 23 | 8,200 | 32.3 | 2.3 | 60.0 | 1.0 | 0.0 | 4.3 | 34.6 | 2,837 |
| | 8,900 | 35.6 | 3.3 | 53.3 | 2.0 | 1.0 | 4.6 | 39.0 | 3,471 |
| 24 | 12,778 | 25.0 | 1.0 | 69.6 | 1.6 | 0.0 | 2.6 | 26.0 | 3,322 |
| | 9,575 | 40.8 | 1.6 | 51.5 | 4.2 | 1.3 | 0.5 | 42.4 | 4,059 |
| 25 | 10,667 | 25.3 | 1.6 | 70.6 | 0.0 | 0.6 | 1.6 | 27.0 | 2,880 |
| | 12,550 | 38.3 | 1.3 | 56.3 | 1.0 | 1.0 | 2.0 | 39.6 | 4,970 |
| Average.... | 9,482 | 23.9 | 3.1 | 65.7 | 2.6 | 0.6 | 3.1 | 27.4 | 2,574 |
| | 9,054 | 32.7 | 2.9 | 57.2 | 2.8 | 0.4 | 3.8 | 35.7 | 3,338 |

decrease was 16.3 per cent. Table III gives 0.0 as the average percentile change in the five persons included there, while the average total lymphocyte increase in the same group was 3.5 per cent.

TABLE VII.

*Complete Counts on Individuals Showing a Decrease in Lymphocytes after Sunburn.*

| Individual No. | White blood corpuscles. | Small lymphocytes. | Large lymphocytes. | Polymorphonuclear neutrophils. | Polymorphonuclear eosinophils. | Polymorphonuclear basophils. | Transitionals and large mononuclears. | Lymphocytes. | Absolute lymphocytes. |
|---|---|---|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| 26 | 8,683 | 29.9 | 4.8 | 58.9 | 0.6 | 0.7 | 5.0 | 34.6 | 2,973 |
| | 8,933 | 28.8 | 3.4 | 62.0 | 1.1 | 0.5 | 4.1 | 32.2 | 2,768 |
| 27 | 6,714 | 36.8 | 5.5 | 46.5 | 2.3 | 1.8 | 7.0 | 42.3 | 2,874 |
| | 7,400 | 30.3 | 2.6 | 62.6 | 0.0 | 1.0 | 3.3 | 32.0 | 2,442 |
| 28 | 6,249 | 30.5 | 5.0 | 58.6 | 1.3 | 0.4 | 4.2 | 35.5 | 2,199 |
| | 8,658 | 23.8 | 4.3 | 64.6 | 2.2 | 0.6 | 4.5 | 28.0 | 2,353 |
| 29 | 7,900 | 37.0 | 4.3 | 49.6 | 1.6 | 0.3 | 7.0 | 41.3 | 3,263 |
| | 10,250 | 26.3 | 3.2 | 59.5 | 3.0 | 0.6 | 7.2 | 29.1 | 2,972 |
| 30 | 12,750 | 37.0 | 5.0 | 55.0 | 0.6 | 0.3 | 2.0 | 42.0 | 5,355 |
| | 13,000 | 34.0 | 2.3 | 59.3 | 1.0 | 0.3 | 3.0 | 36.3 | 4,719 |
| 31 | 11,100 | 39.6 | 2.6 | 53.3 | 0.6 | 0.0 | 1.6 | 42.3 | 4,695 |
| | 9,050 | 36.0 | 1.6 | 56.6 | 0.0 | 0.3 | 5.3 | 37.6 | 3,403 |
| 32 | 6,850 | 39.5 | 4.3 | 47.8 | 1.8 | 1.2 | 5.3 | 43.8 | 3,000 |
| | 6,783 | 40.5 | 2.2 | 51.2 | 2.9 | 0.4 | 2.6 | 42.7 | 2,917 |
| 33 | 8,950 | 31.6 | 3.0 | 57.6 | 3.3 | 0.0 | 4.3 | 34.6 | 3,097 |
| | 8,950 | 28.3 | 4.3 | 63.0 | 2.3 | 0.0 | 2.0 | 32.6 | 2,918 |
| Average.... | 8,650 | 35.2 | 4.3 | 53.4 | 1.5 | 0.6 | 4.6 | 39.6 | 3,807 |
| | 9,128 | 31.0 | 3.0 | 59.9 | 1.7 | 0.5 | 4.0 | 33.8 | 3,187 |

As may be seen from Tables VI, VII, and VIII there was no constant change in the total numbers of white blood corpuscles per c.mm. of blood as determined before and after exposure. A study of these tables also shows that the polymorphonuclear neutrophilic leucocytes tended to vary in inverse proportion with the lympho-

cytes, these cells decreasing when the lymphocytes increased and *vice versa*. The polymorphonuclear eosinophilic and basophilic (mast) cells and the group composed of the transitional and large mononuclear granular cells (bone marrow oxidase cells, Evans[8]) remained very constant.

TABLE VIII.

*Complete Counts on Individuals Showing No Change in Lymphocytes after Sunburn.*

| Individual No. | White blood corpuscles. | Small lymphocytes. | Large lymphocytes. | Polymorphonuclear neutrophils. | Polymorphonuclear eosinophils. | Polymorphonuclear basophils. | Transitionals and large mononuclears. | Lymphocytes. | Absolute lymphocytes. |
|---|---|---|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| 34 | 15,600 | 26.3 | 3.0 | 63.6 | 1.0 | 1.0 | 5.0 | 29.3 | 4,571 |
| | 17,800 | 25.3 | 4.6 | 61.6 | 1.6 | 0.6 | 6.0 | 30.0 | 5,340 |
| 35 | 7,900 | 28.3 | 5.0 | 61.6 | 0.6 | 0.3 | 4.0 | 33.3 | 2,631 |
| | 7,850 | 31.0 | 2.0 | 61.3 | 1.3 | 0.6 | 3.6 | 33.0 | 2,591 |
| 36 | 11,350 | 14.3 | 3.6 | 77.0 | 2.0 | 1.0 | 2.0 | 18.0 | 2,043 |
| | 13,950 | 15.3 | 2.3 | 74.3 | 0.6 | 0.6 | 6.6 | 17.6 | 2,455 |
| 37 | 11,975 | 24.9 | 2.5 | 68.3 | 0.5 | 0.8 | 2.8 | 27.5 | 3,378 |
| | 9,750 | 24.7 | 2.1 | 67.4 | 2.4 | 0.5 | 2.6 | 26.9 | 2,600 |
| 38 | 7,900 | 24.3 | 2.6 | 61.6 | 6.0 | 0.0 | 5.3 | 27.0 | 2,133 |
| | 7,825 | 23.3 | 4.5 | 62.2 | 5.0 | 0.5 | 4.5 | 27.8 | 2,213 |
| Average.... | 10,945 | 23.6 | 3.4 | 66.4 | 2.0 | 0.6 | 3.8 | 27.0 | 2,951 |
| | 11,435 | 23.9 | 3.1 | 65.4 | 2.2 | 0.6 | 4.7 | 27.1 | 3,060 |

Rollier and others[9] have emphasized the fact that tanning is necessary to obtain beneficial results in tuberculosis with heliotherapy. In this connection it is interesting to note that of the thirteen individuals who showed no increase in their circulating lymphocytes, six had the pasty type of skin which does not tan. Three others were so dark normally that it was impossible to determine whether or not there had been additional pigmentation. Five

[8] Evans, F. A., *Arch. Int. Med.*, 1916, xviii, 692.

[9] Rollier, A., etc., quoted in Roatta,[5] p. 19.

of the eight persons whose blood showed a loss in lymphocyte percentage had circulating cells of this type in excess of 40 per cent before exposure to the sun. These observations are interesting as offering possible explanations of the apparent exceptions to the general tendency to develop a definite lymphocytosis after chronic solar dermatitis. Thus the twenty-five persons with a definite increase in circulating lymphocytes were all tanned to a greater or less extent. The increase in lymphocytes, moreover, tended to be greatest in the individuals in whom the pigmentation was most increased, yet this was not invariably the case; so that the increase in lymphocytes and degree of tanning did not always go hand in hand so far as this series of tests is concerned.

In regard to complexion, no particular difference between the blood or skin response to solar irritation was noted with the exception of the three individuals in whom it was impossible to determine whether or not additional pigmentation was present. So far as observed age and sex had no effect on the blood response to light.

## DISCUSSION.

From the results recorded here it seems that the rays contained in the solar spectrum, as it reaches the earth in the temperate zone in midsummer, bring about changes in the white corpuscles of the blood in a manner similar to that recognized as characteristic of the x-rays. The manner of action is equally obscure with regard to both agents.

Of interest in connection with this study are blood counts made by Chamberlain and Vedder[10] on Americans living in the Philippines. Although analyzed by them to ascertain the effect of the tropical climate on the Arneth blood picture, their counts show a higher percentage of lymphocytes than normal individuals in the temperate zone.[11] In the 72 cases reported there was an average of 38.6 per cent. The tropical service of the soldiers composing the series averaged 28.7

[10] Chamberlain, W. P., and Vedder, E. B., *Philippine. J. Sc.*, 1911, vi, 408.

[11] In a series of more than 100 observations on the bloods of 46 healthy individuals from 20 to 65 years of age, made in connection with this study, it was found that the lymphocytes averaged 29.3 per cent.

months. Wickline,[12] in counts made upon the blood of 104 soldiers 3, 12, and 19 months after arrival in the Philippines, showed that there was a definite and progressive increase in the mononuclear cells. These cells averaged 31.4 per cent at the first, 34.4 per cent at the second, and 39.5 per cent at the third determination. Although there is no distinction made between the mononuclear elements, tending to separate the lymphocytes from the cells belonging to the granular series, some idea of the direction in which the cells of lymphatic origin varied may be obtained by following the percentage of small mononuclear cells, which are undoubtedly the small lymphocytes. The average percentage of these cells at the first count was 21.8, at the second count 26.6, and at the third count 33.3. Disregarding the large lymphocytes, which should be combined with the small cells of the same series to give an accurate idea of their number at each determination, it can be seen that there was a definite increase of these cells in the blood after 19 months in the tropical climate. The soldiers were undoubtedly exposed to the sun for long periods of time. It also seems possible that the invisible spectrum is somewhat widened in the tropics[13] and there is consequently a wider range of ultra-violet rays than in the temperate zone. The intensity of the visible rays of the spectrum is also increased as we approach the equator. It is probable that the invisible rays increase likewise in proportion to those that are visible. Although the effect on the blood seems to be related to the ultra-violet rays of sunlight, it is impossible to rule out the effect of infra-red, or heat waves in these observations.

## SUMMARY.

Chronic solar dermatitis was accompanied, in 25 of the 38 individuals studied, by an appreciable increase, percentage and absolute, in the number of circulating lymphocytes.

In eight there was a definite decrease and in five no appreciable change after prolonged exposure to the rays of the summer sun.

Of the thirteen subjects with no increase in blood lymphocytes, six

[12] Wickline, W. A., *Mil. Surg.*, 1908, xxiii, 282.

[13] Bovie, W. T., *Am. J. Trop. Dis. and Prev. Med.*, 1914–15, ii, 512.

failed to tan, three were so dark originally that to determine an increase was impossible, and five had an extremely high lymphocyte count at the first count.

Blood counts on white persons living in the Philippines indicate that the blood lymphocytes are likewise increased after a prolonged period of residence in the tropical zone.

Because of the parallelism between the tanning and the blood changes it seems probable that the lymphocytosis observed in the majority of instances, which is similar to the response of the blood of animals to small doses of the x-rays, is due to the effects of the ultra-violet rays contained in the solar spectrum.

# STUDIES ON X-RAY EFFECTS.

## I. Destructive Action on Blood Cells.*

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Received for publication, September 16, 1918.)

Pusey,[1] Senn,[2] Brown,[3] Bryant and Crane,[4] and others,[5,6] have reported on cases of leucemia treated with the x-rays and all seem to agree that there is a definite reduction of the circulating leucocytes, following treatment. Most of the cases reported were, however, of the splenomyelogenous type and the myelocytes were the cells most affected. Capps and Smith[7] treated lymphatic leucemia with the Roentgen rays and noted a marked reduction of the circulating lymphocytes, after treatment, in five of their six cases.

Heineke,[8] the first to make careful histological examinations of animals following x-ray exposures, demonstrated that the lymphatic tissues of the body were primarily affected. He found degeneration of the lymph follicles in the spleen and lymph glands and a diminution of circulating lymphocytes. He correlated his observations by suggesting that the diminution in circulating lymphocytes is directly referable to the selective destruction of the lymphogenic tissues by the x-rays.

Helber and Linser[9] published blood counts made on a rabbit before and after x-ray treatment. A marked percentile and absolute reduction in the circulating

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*This investigation was carried out by means of funds from the Rutherford Donation.

[1] Pusey, W. A., *J. Am. Med. Assn.*, 1902, xxxviii, 166.

[2] Senn, N., *N. Y. Med. J.*, 1903, lxxvii, 665.

[3] Brown, E. J., *J. Am. Med. Assn.*, 1904, xlii, 827.

[4] Bryant, B. L., and Crane, H. H., *Med. Rec.*, 1904, lxv, 574.

[5] For a review of the literature see Warthin, A. S., *Internat. Clin.*, 1906, iv, series 15, 243.

[6] For a further review of the literature see Pancoast, H. K., *Univ. Penn. Med. Bull.*, 1906–07, xix, 282.

[7] Capps, J. A., and Smith, J. F., *J. Am. Med. Assn.*, 1904, xliii, 981.

[8] Heineke, H., *Mitt. Grenzgeb. Med. u. Chir.*, 1905, xiv, 21.

[9] Helber, E., and Linser, P., *Münch. med. Woch.*, 1905, lii, 689.
Linser, P., and Helber, E., *Deutsch. Arch. klin. Med.*, 1905, lxxxiii, 479.

lymphocytes occurred. These cells, before treatment, represented 32 per cent of the white blood corpuscles, a total of 2,080 lymphocytes per c.mm. of blood. After treatment they had decreased to 6 per cent, or but 120 cells per c. mm. In a white rat there was a reduction of lymphocytes from 60 per cent, or 8,400 cells before, to 30 per cent, or 1,020 cells after x-ray treatment. These results were confirmed by other experiments.

Warthin[5] confirmed Heineke's work and added further evidence indicating that the x-rays have a specific destructive action on lymphoid tissues. He found the Malpighian bodies of the spleen to be first affected and later the lymph glands and bone marrow. Histologically, he noted fragmentation of the tissue lymphocytes, the particles being ingested by phagocytes. Mitotic figures were absent or very infrequently encountered in the lymphogenic tissues following x-ray treatment. After a short interval the lymph follicles, when present, were either inconspicuous or invisible in gross, and but few lymphocytic elements were found microscopically. The stroma of the organs affected was much in evidence, owing to the destruction and consequent disappearance of the lymphogenic cells. Heineke and Warthin both employed x-rays in lethal doses.

It has been shown that resistance to cancer[10] and tuberculosis,[11] in animals, is attended by an increase in number of the circulating lymphocytes and that the resistance, as well as the lymphocytosis, may be destroyed by x-rays in proper dosage.[11, 12] Likewise the natural resistance of animals to heteroplastic tissue grafts, which seems to be associated definitely with a local accumulation of lymphocytes, may be destroyed by the x-rays.[13]

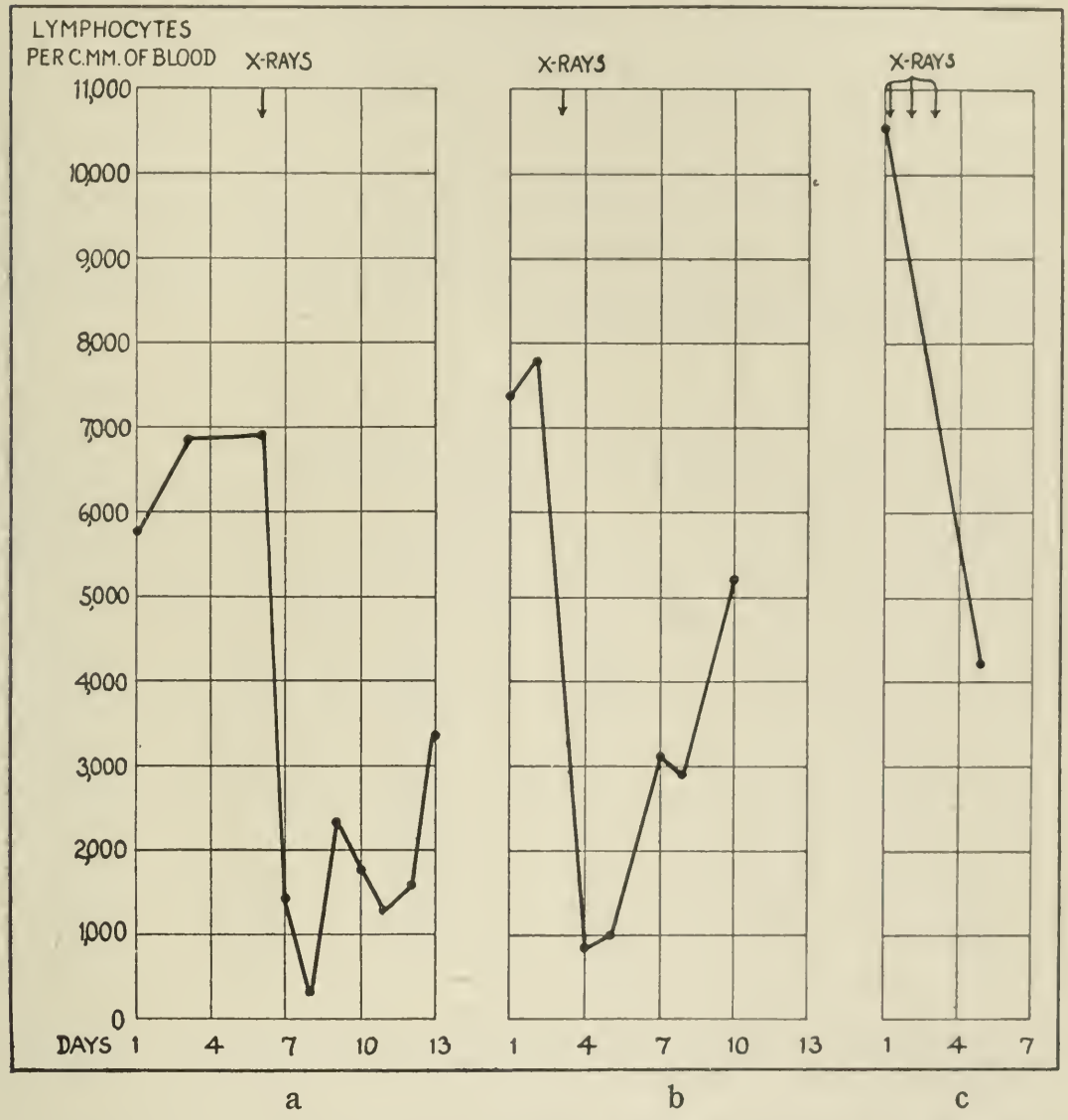
Inasmuch as (*a*) it is not possible to estimate the dosage employed in most of the older experiments concerning the effect of the x-rays on the blood, because gas tubes were used, (*b*) only a few blood counts have been published in these cases and their significance has not been adequately explained, (*c*) the x-rays are now being used with increasing frequency in therapeusis; it seemed important to obtain accurate information regarding the response of the blood to x-rays, and with this purpose the following results are recorded.

## EXPERIMENTAL.

*Experiment 1.*—Eight areas, comprising both flanks of a Shetland pony, about 8 years old, were successively exposed in a single day to unfiltered x-rays generated by a Coolidge tube. The factors governing the dose at each exposure were:

[10] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.
[11] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.
[12] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.
[13] Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459.

spark-gap 8 inches, milliamperes 5, distance from the target to the skin 12 inches, and time 10 minutes. By computing the dose[14] from the factors given, it was found that the animal received over each area 20, or over the eight areas 160 Holzknecht




TEXT-FIG. 1, *a*, *b*, and *c*. (*a*) The effect of x-ray treatment on the circulating lymphocytes of a pony. (*b*) The effect of x-ray treatment on the circulating lymphocytes of a cat. (*c*) The effect of x-ray treatment on the circulating lymphocytes of a guinea pig.

units. The total number of lymphocytes per c. mm. of blood at each observation, during the period of study, is charted in Text-fig. 1, *a*. The per cent

[14] Remer, J., and Witherbee, W. D., *Am. J. Roentgen.*, 1917, iv, 303.

and total number of lymphocytes and polymorphonuclear neutrophilic leucocytes per c. mm. of blood at each determination are tabulated in Table I.

*Experiment 2.*—A healthy adult monkey (*Macacus rhesus*) was given a series of unfiltered x-ray treatments, extending over a period of 8 days. Seven doses were given, the dorsal and ventral surfaces of the body being alternately exposed. The factors at each treatment were: spark-gap 3 inches, milliamperes 10, distance from the target of the Coolidge tube to the skin 12 inches, and time 4 minutes. This dose represents, at each exposure 6, or a total of 42 Holzknecht units. Text-fig. 2 graphically illustrates the effect of the x-rays on the circulating lymphocytes in this animal, each determination referring to the total number of lymphocytes per c. mm. of blood. In Table II the blood counts, made at different times in the course of the experiment, are summarized to show more fully the changes in the circulating cells following treatment.

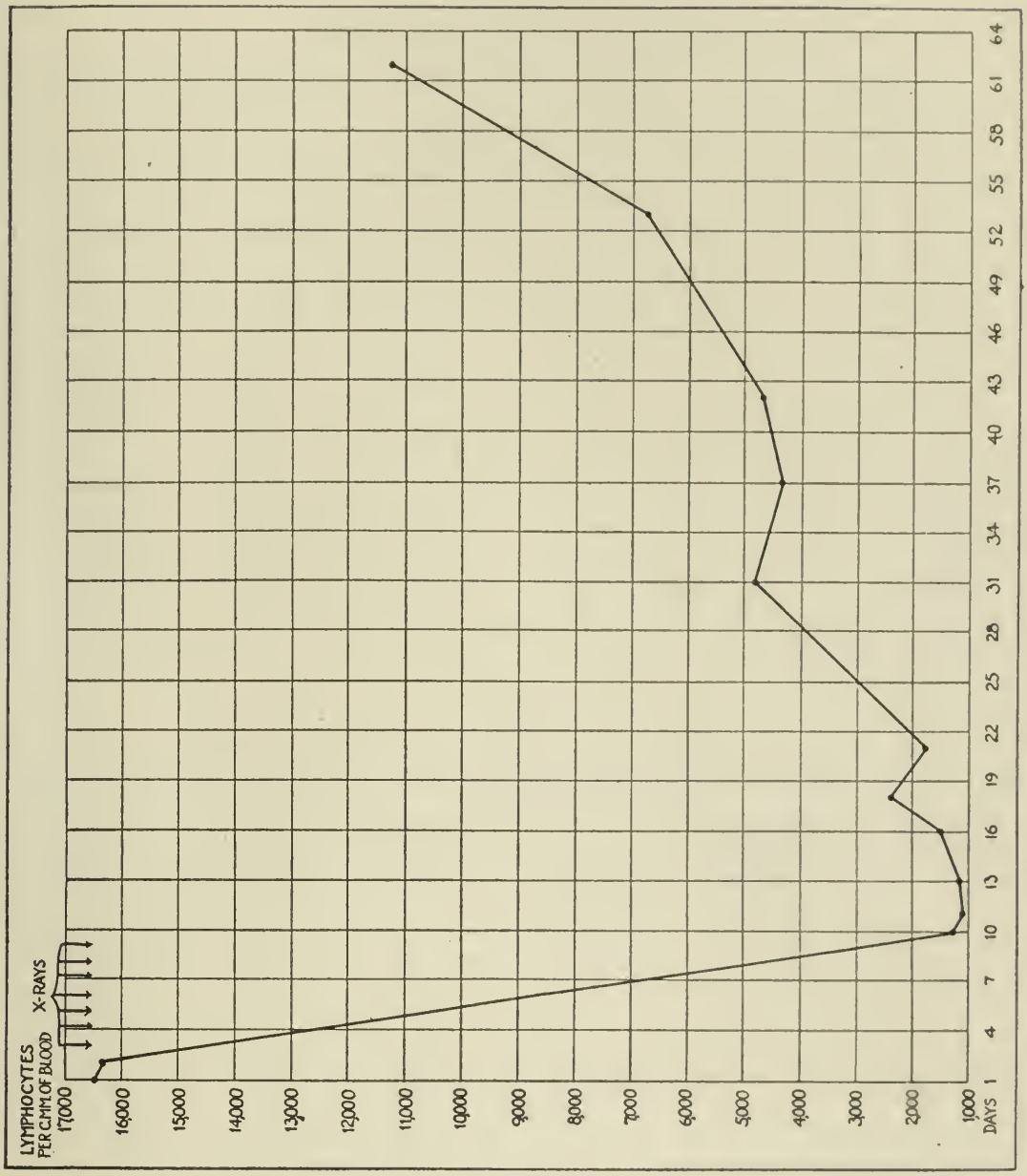
TABLE I.

*Pony Receiving 160 Holzknecht Units.*

| Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|
| | | Per cent. | Total No. | Per cent. | Total No. |
| | *days* | | | | |
| 1 | | 43.7 | 5,790 | 55.7 | 7,380 |
| 3 | | 48.3 | 6,859 | 48.3 | 6,859 |
| 6 | | 44.2 | 6,906 | 50.0 | 7,813 |
| 7 | 1 | 6.3 | 1,473 | 93.7 | 21,902 |
| 8 | 2 | 1.2 | 321 | 92.7 | 24,797 |
| 9 | 3 | 11.7 | 2,404 | 84.7 | 17,406 |
| 10 | 4 | 24.2 | 1,785 | 66.7 | 4,918 |
| 11 | 5 | 29.3 | 1,282 | 60.3 | 2,638 |
| 12 | 6 | 27.0 | 1,593 | 66.3 | 3,912 |
| 13 | 7 | 31.6 | 3,410 | 64.0 | 6,912 |

*Experiment 3.*—A full grown cat was given two treatments in a single day with unfiltered x-rays emitted by a Coolidge tube. One exposure was made on the dorsal and one on the ventral surface of the body. The factors at each exposure were: spark-gap 3 inches, milliamperes 10, distance from the target to the skin 12 inches, and time 1 minute. The dose, in this instance, was for each exposure 1½, or a total of 3 Holzknecht units. Text-fig. 1, *b* is a curve showing the total number of lymphocytes plotted against the days on which blood counts were made. Table III gives percentages and actual numbers of white blood cells on the days recorded in Text-fig. 1, *b*.

*Experiment 4.*—A guinea pig was exposed to unfiltered x-rays on 3 successive days, after an initial blood count had been made. On the 5th day, 48 hours after the last x-ray treatment, a second count was made; Text-fig. 1, *c* shows




TEXT-FIG. 2. The effect of x-ray treatment on the circulating lymphocytes of a *Macacus rhesus*.

graphically the fall in the total number of lymphocytes per c. mm. of blood following the administration of the x-rays. Table IV gives the actual figures determined at the two blood examinations. The factors at each treatment were:

TABLE II.

*Monkey Receiving 42 Holzknecht Units.*

| Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|
| | | Per cent. | Total No. | Per cent. | Total No. |
| | *days* | | | | |
| 1 | | 62.7 | 16,537 | 36.0 | 9,495 |
| 2 | | 56.7 | 16,415 | 40.7 | 11,783 |
| 10 | 1 | 4.0 | 1,283 | 94.7 | 30,375 |
| 11 | 2 | 4.7 | 1,128 | 95.0 | 22,800 |
| 13 | 4 | 7.0 | 1,155 | 92.7 | 15,296 |
| 16 | 7 | 5.7 | 1,526 | 94.0 | 25,169 |
| 18 | 9 | 11.7 | 2,463 | 88.0 | 18,524 |
| 21 | 12 | 7.0 | 1,787 | 92.3 | 23,560 |
| 28 | 19 | 27.0 | 4,846 | 71.0 | 12,745 |
| 37 | 28 | 23.3 | 4,351 | 74.7 | 13,950 |
| 42 | 33 | 31.7 | 4,771 | 64.3 | 9,677 |
| 53 | 44 | 29.0 | 6,779 | 66.7 | 15,598 |
| 63 | 54 | 55.7 | 11,224 | 40.7 | 8,201 |

TABLE III.

*Cat Receiving 3 Holzknecht Units.*

| Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|
| | | Per cent. | Total No. | Per cent. | Total No. |
| | *days* | | | | |
| 1 | | 35.0 | 7,389 | 55.7 | 11,759 |
| 2 | | 34.7 | 7,825 | 56.0 | 12,628 |
| 4 | 2 | 5.0 | 844 | 94.0 | 15,876 |
| 5 | 3 | 8.7 | 1,005 | 83.7 | 9,672 |
| 7 | 5 | 16.0 | 3,093 | 77.0 | 14,886 |
| 8 | 6 | 12.0 | 2,907 | 79.0 | 19,144 |
| 10 | 8 | 11.0 | 5,194 | 88.0 | 41,555* |

* Distemper developed later in this cat and the high polymorphonuclear leucocyte count may be due to this.

spark-gap 2¼ inches, milliamperes 10, distance from the target of the Coolidge tube to the skin 12 inches, and time 3 minutes. This dose represents, approximately, 3 Holzknecht units.

In two experiments on mice a gas tube had been used to generate x-rays. The dose was necessarily indefinite and the only measure of the comparative amount of the x-rays received by each animal consists in the constant established by the fact that the mice included in each experiment were exposed simultaneously and for the same length of time. These experiments are included because they demonstrated the tendency of the circulating lymphocytes to decrease in number after animals had been exposed to the x-rays generated by gas tubes and because this decrease was, in many ways, similar to that observed in Experiments 1 to 4. Furthermore, there is a definite relation between the response of the various animals in a series, as determined by blood counts, to x-ray treatment.

TABLE IV.

*Guinea Pig Receiving Approximately 3 Holzknecht Units.*

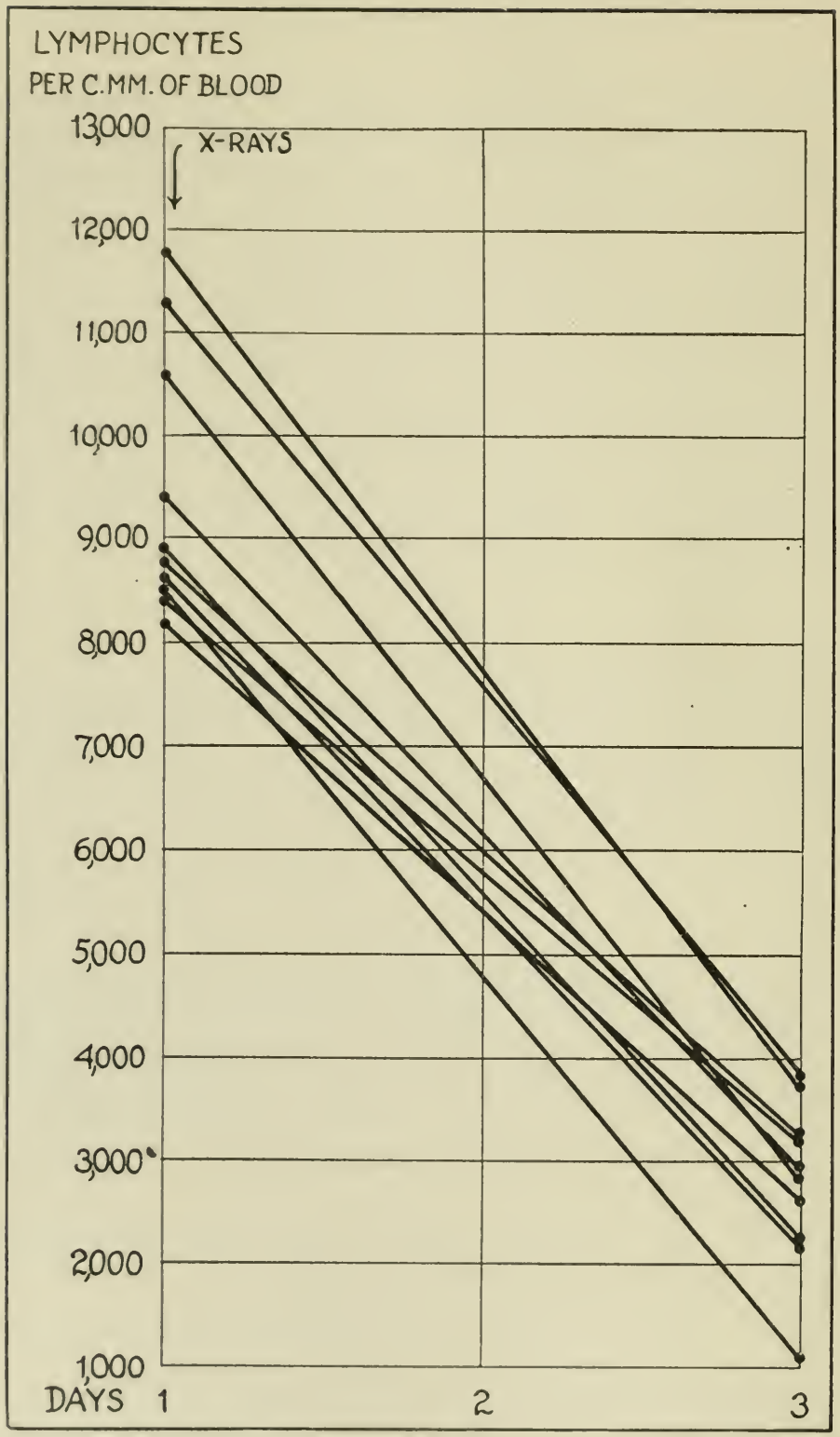
| Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|
| | | Per cent. | Total No. | Per cent. | Total No. |
| | *days* | | | | |
| 1 | | 36.7 | 10,606 | 61.7 | 17,831 |
| 5 | 2 | 16.3 | 4,283 | 81.3 | 21,138 |

*Experiment 5.*—Ten normal, adult, white rats were simultaneously exposed for the same length of time to unfiltered x-rays generated by a rather soft gas tube. Total white and differential blood counts were made on each animal immediately before and 48 hours after exposure. The effect of the x-rays on the circulating lymphocytes (total number per c. mm.) is shown in Text-fig. 3, where each line represents the difference, in a single rat, between the number of lymphocytes before and after treatment.

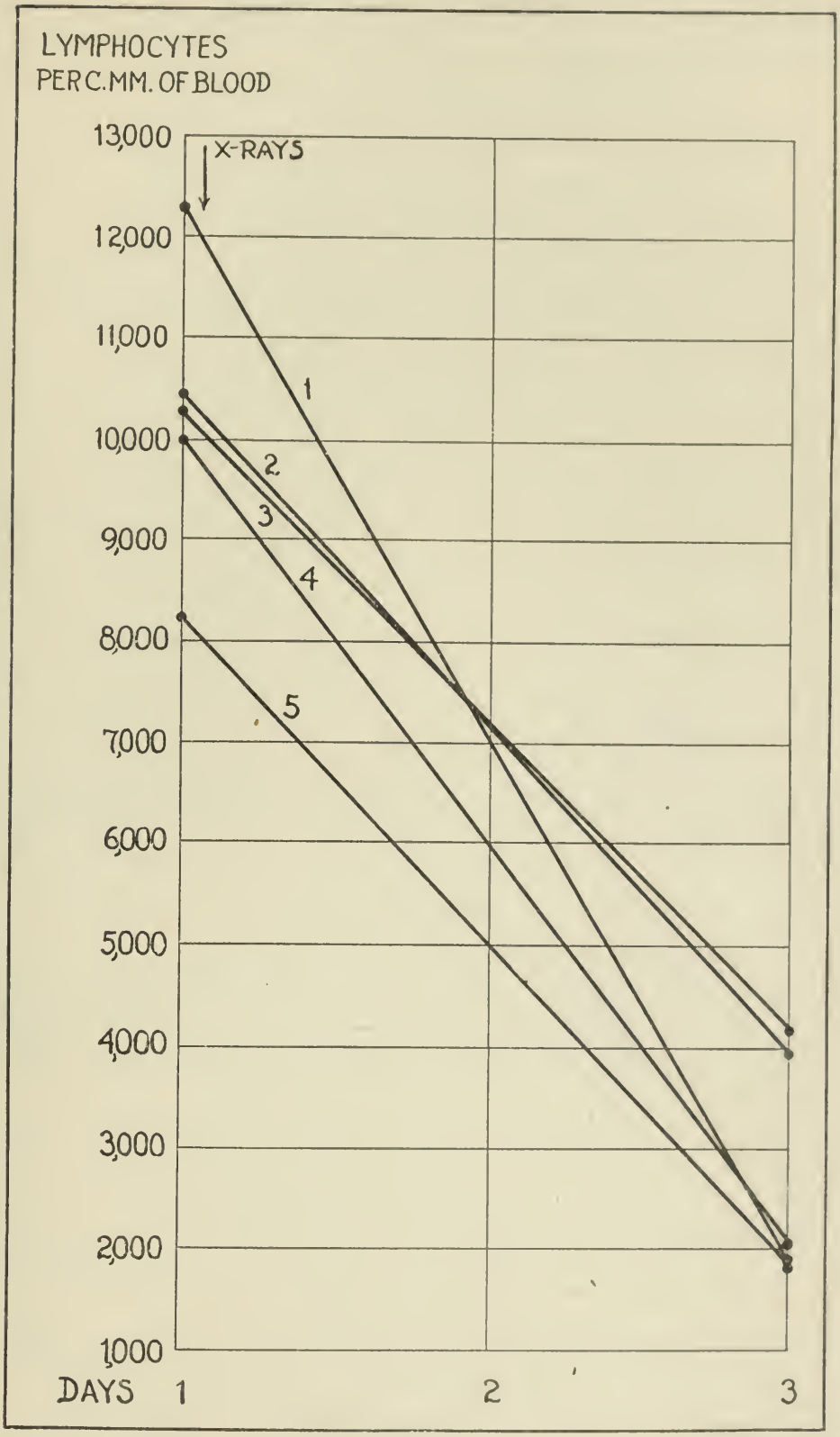
*Experiment 6.*—The procedure followed in Experiment 5 was repeated with five additional white mice and the results are shown in Text-fig. 4, in which the lines have the same significance as those in Text-fig. 3.

*Experiment 7.*—Four mice were simultaneously given seven treatments with unfiltered x-rays, the Coolidge tube being used. Blood counts were made on each animal before the first and 48 hours after the last x-ray exposure. The effect of the x-rays on the blood of each mouse is shown in the usual manner in Text-fig. 5; additional data of interest are given in Table V.[15] The factors at

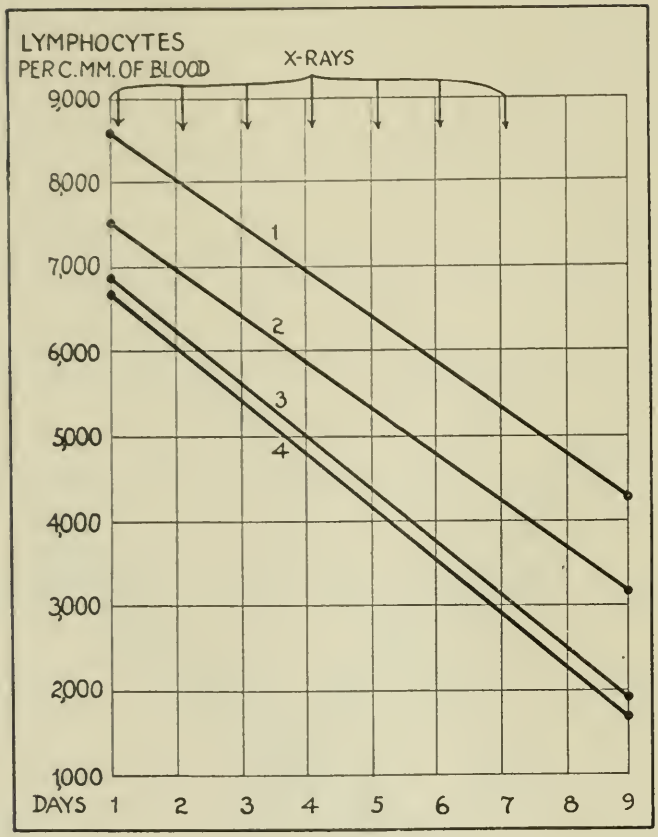
[15] The number of the animal in the curve and in the table corresponds, in this and the following experiments.




TEXT-FIG. 3. The effect of x-ray treatment on the circulating lymphocytes of ten white rats. In this and the following text-figures each line represents the difference in a single animal between the number of lymphocytes before and after treatment.




TEXT-FIG. 4. The effect of x-ray treatment on the circulating lymphocytes of five white mice.



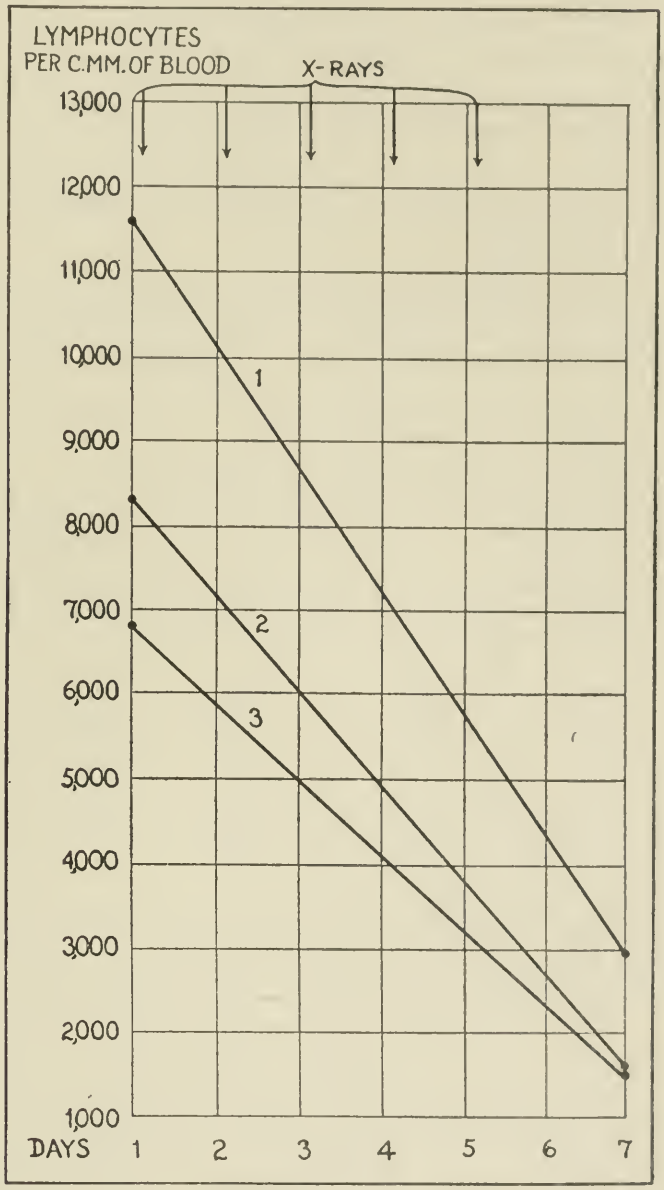

TEXT-FIG. 5. The effect of x-ray treatment on the circulating lymphocytes of four white mice.

TABLE V.

*Four Mice Receiving 14 Holzknecht Units.*

| Mouse No. | Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|---|
| | | | Per cent. | Total No. | Per cent. | Total No. |
| | | *days* | | | | |
| 1 | 1 | | 73.0 | 8,588 | 27.0 | 3,180 |
| | 9 | 2 | 55.3 | 4,301 | 44.7 | 3,477 |
| 2 | 1 | | 90.3 | 7,527 | 9.6 | 800 |
| | 9 | 2 | 57.0 | 3,103 | 43.0 | 2,341 |
| 3 | 1 | | 48.2 | 6,909 | 51.7 | 7,410 |
| | 9 | 2 | 42.0 | 1,914 | 58.0 | 2,643 |
| 4 | 1 | | 51.0 | 6,687 | 49.0 | 6,424 |
| | 9 | 2 | 14.0 | 1,680 | 86.0 | 10,320 |

each exposure were: spark-gap 2¼ inches, milliamperes 10, distance from the target to the skin 12 inches, and time of exposure 2 minutes. This represents 2, at a single exposure, or a total of 14 Holzknecht units.




TEXT-FIG. 6. The effect of x-ray treatment on the circulating lymphocytes of three adult white rats.

*Experiment 8.*—Three adult white rats were exposed to the unfiltered x-rays generated by a Coolidge tube. The factors were: spark-gap 3 inches, milliamperes 10, distance from the target to the skin 12 inches, and time 4 minutes. This dose was repeated daily for 5 days. Each exposure represents 6, a total of

30 Holzknecht units being therefore given. White and differential blood counts made before the first and 48 hours after the last exposure are summarized in Table VI and total lymphocytes per c. mm. of blood at each determination charted in Text-fig. 6.

*Experiment 9.*—Three rabbits, after preliminary blood counts, were given seven daily, unfiltered doses of x-rays, each dose depending on the following factors: spark-gap 3 inches, milliamperes 10, distance from the target to the skin 12 inches, and time 4 minutes. This represents 6 at a single dose, or a total of 42 Holzknecht units. The dorsal and ventral surfaces of the animals were exposed to the rays on alternate days. Text-fig. 7 and Table VII refer to the blood counts on these animals.

*Experiment 10.*—Two rabbits were each given a single treatment with the unfiltered x-rays emitted by a Coolidge tube, which was controlled by the following factors: spark-gap 3 inches, milliamperes 20, distance from the target to the

TABLE VI.

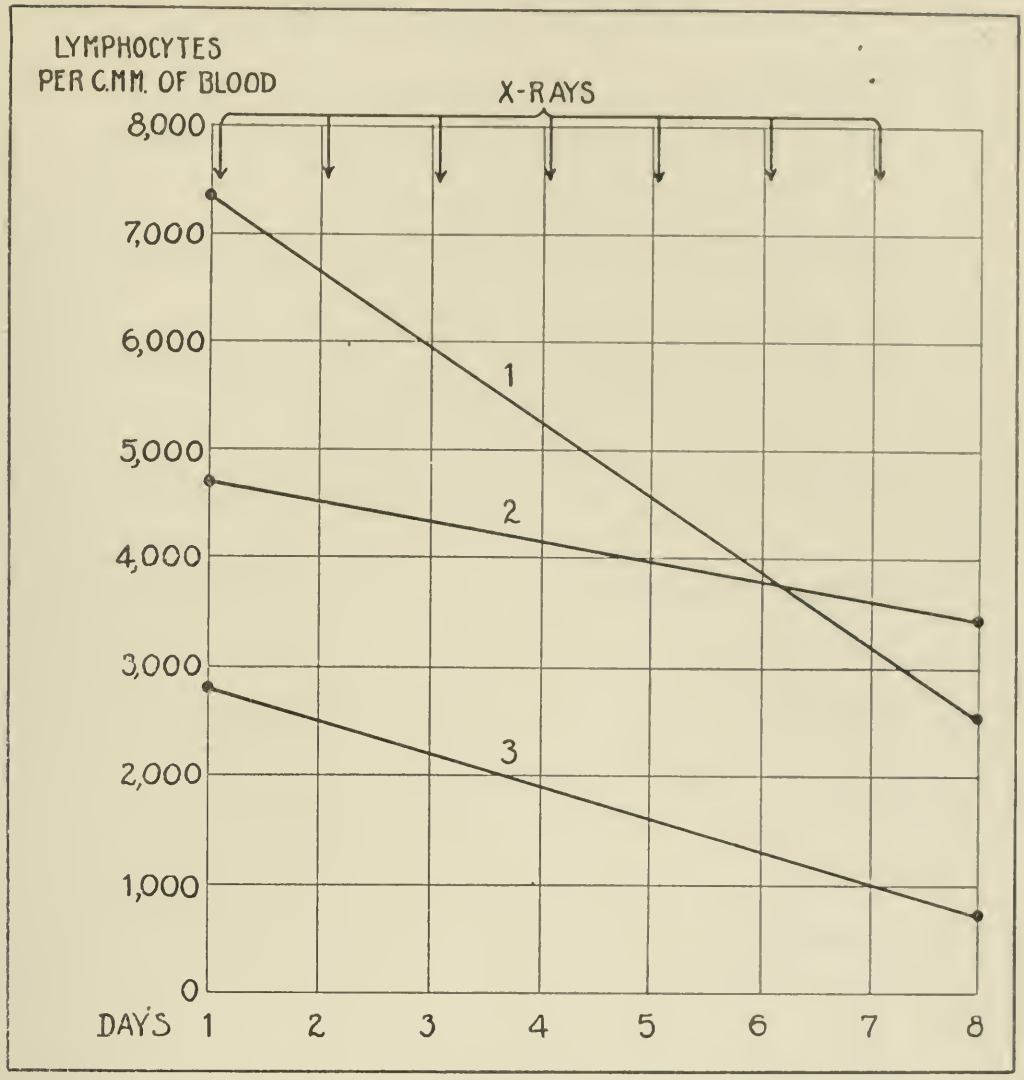
*Three Rats Receiving 30 Holzknecht Units.*

| Rat No. | Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|---|
| | | | Per cent. | Total No. | Per cent. | Total No. |
| | | *days* | | | | |
| 1 | 1 | | 89.7 | 11,683 | 10.3 | 1,342 |
| | 7 | 2 | 62.3 | 2,990 | 37.3 | 1,790 |
| 2 | 1 | | 63.0 | 8,096 | 37.0 | 4,755 |
| | 7 | 2 | 16.3 | 1,593 | 83.7 | 8,182 |
| 3 | 1 | | 79.2 | 6,831 | 20.7 | 1,785 |
| | 7 | 2 | 48.7 | 1,583 | 51.3 | 1,667 |

skin 12 inches, and time 4 minutes. This dose represents 11 Holzknecht units. Text-fig. 8 and Table VIII refer to the blood counts on these animals.

*Experiment 11.*—Two rabbits at a single exposure were treated with x-rays filtered through 3 mm. of aluminum, the dose approximating 5 Holzknecht units. This was determined by the following factors: spark-gap 9 inches, milliamperes 5, distance from the target to the skin 12 inches, and time 5 minutes and 20 seconds. Text-fig. 9 and Table IX refer to the circulating lymphocytes and polymorphonuclear leucocytes in these animals, before and 48 hours after exposure.

*Experiment 12.*—Three monkeys were each exposed to the unfiltered x-rays generated by a Coolidge tube for a total of seven treatments. Monkeys 1 and 2 had daily exposures, while Monkey 3 was given the seven treatments in 4 days. The factors at each treatment were: spark-gap 3 inches, milliamperes 10, distance from the target to the skin 12 inches, and time 4 minutes. At each exposure 6, or a total of 42 Holzknecht units were therefore given to each animal. Text-fig. 10 and Table X give the data for these monkeys.

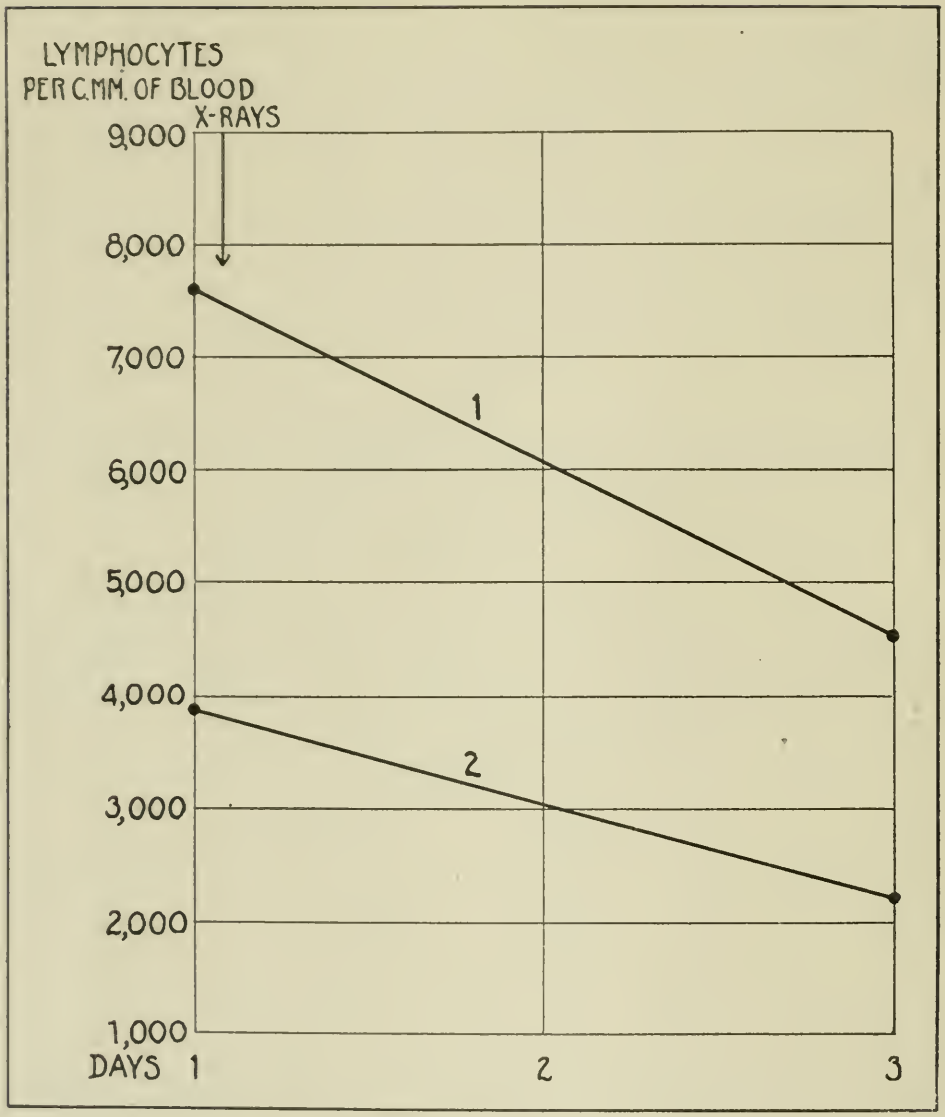



TEXT-FIG. 7. The effect of x-ray treatment on the circulating lymphocytes of three rabbits.

TABLE VII.

*Three Rabbits Receiving 42 Holzknecht Units.*

| Rabbit No. | Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|---|
| | | | Per cent. | Total No. | Per cent. | Total No. |
| | | *days* | | | | |
| 1 | 1 | | 55.3 | 7,383 | 42.0 | 5,607 |
| | 7 | 2 | 54.3 | 2,570 | 39.3 | 1,870 |
| 2 | 1 | | 44.7 | 4,716 | 47.7 | 5,032 |
| | 7 | 2 | 39.7 | 3,462 | 55.7 | 4,857 |
| 3 | 1 | | 29.7 | 2,866 | 64.0 | 6,176 |
| | 7 | 2 | 24.3 | 758 | 66.7 | 2,081 |

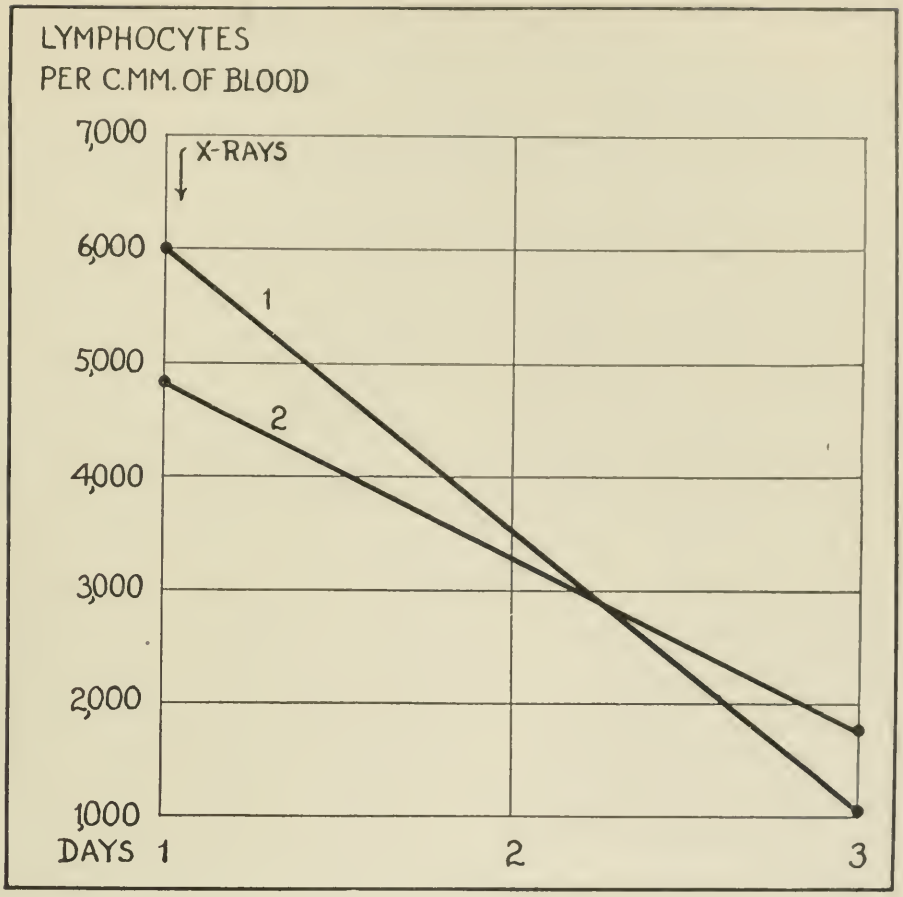



TEXT-FIG. 8. The effect of x-ray treatment on the circulating lymphocytes of two rabbits.

TABLE VIII.

*Two Rabbits Receiving 11 Holzknecht Units.*

| Rabbit No. | Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|---|
| | | | Per cent. | Total No. | Per cent. | Total No. |
| | | *days* | | | | |
| 1 | 1 | | 66.3 | 7,591 | 31.3 | 3,584 |
| | 3 | 2 | 54.7 | 4,568 | 41.3 | 3,449 |
| 2 | 1 | | 51.2 | 3,878 | 48.0 | 3,636 |
| | 3 | 2 | 32.3 | 2,196 | 65.6 | 4,461 |

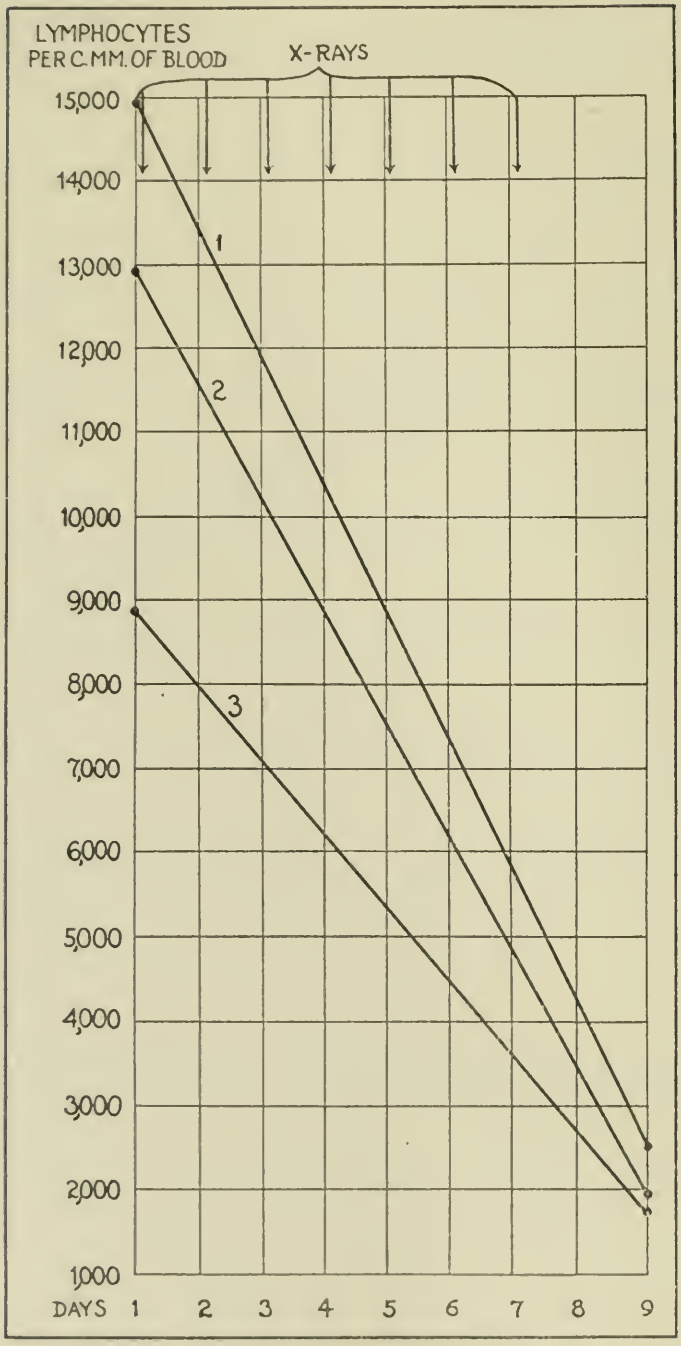



TEXT-FIG. 9. The effect of x-ray treatment on the circulating lymphocytes of two rabbits. The x-rays were filtered through 3 mm. of aluminum.

TABLE IX.

*Two Rabbits Receiving Approximately 5 Holzknecht Units (Filtered).*

| Rabbit No. | Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|---|
| | | | Per cent. | Total No. | Per cent. | Total No. |
| | | *days* | | | | |
| 1 | 1 | | 87.3 | 6,002 | 11.0 | 756 |
| | 3 | 2 | 44.7 | 1,017 | 50.7 | 1,153 |
| 2 | 1 | | 54.0 | 4,860 | 39.7 | 3,573 |
| | 3 | 2 | 27.2 | 1,761 | 65.0 | 4,209 |




TEXT-FIG. 10. The effect of x-ray treatment on the circulating lymphocytes of three monkeys.

TABLE X.

*Three Monkeys Receiving 42 Holzknecht Units.*

| Monkey No. | Day of experiment. | Length of time after x-rays. | Lymphocytes. | | Polymorphonuclear cells. | |
|---|---|---|---|---|---|---|
| | | | Per cent. | Total No. | Per cent. | Total No. |
| | | *days* | | | | |
| 1 | 1 | | 59.7 | 14,925 | 34.3 | 8,575 |
| | 9 | 2 | 17.7 | 2,509 | 77.0 | 10,915 |
| 2 | 1 | | 54.0 | 12,960 | 42.0 | 10,080 |
| | 9 | 2 | 15.0 | 1,980 | 77.5 | 10,230 |
| 3 | 1 | | 54.7 | 8,861 | 43.7 | 7,079 |
| | 6* | 22 | 17.7 | 1,744 | 81.3 | 8,008 |

* In this animal the seven treatments were given in 4 days.

## DISCUSSION.

The immediate effect of the x-rays, in the dose employed in these experiments, is a sudden decrease in the circulating lymphocytes, evident in every curve and table in the series. The curves all represent total numbers of lymphocytes, small and large varieties combined, per c. mm. of blood. When the lymphocytes are studied in terms of percentage of total white blood cells the results are not so striking, and, while in most instances there is a definite fall in percentage as well as in actual numbers of these cells after x-ray treatment, an occasional instance is encountered where the percentile change is slight or absent. For example, whereas the total lymphocytes of Rabbit 1, Table VII, decreased from 7,383 before to 2,570 after exposure, the corresponding fall in percentage of lymphocytes was small; *i.e.*, from 55.3 to 54.3 per cent. The latter fall is well within the limits of counting error and is therefore negligible. It seems, therefore, that an estimation of the total number of lymphocytes per c. mm. of blood, determined by multiplying the total white count by the combined percentage of large and small lymphocytes, before and after x-ray treatment offers a more accurate indication of the effect on the blood than can be determined by following percentage figures only. This means, in other terms, that while the x-rays, in most instances, affect the lymphocytes selectively, occasional cases occur in which together with the lymphocytes the granular blood cells are also destroyed.

When the total number of circulating lymphocytes is plotted against the time in days a curve is formed which reaches its lowest level 48 hours after the administration of the x-rays (Text-figs. 1, *a*, *b*, and 2). Following this fall there is a primary rise, which reaches its acme from 3 to 5 days after the last x-ray exposure (3 days in Experiment 1, 9 days in Experiment 2, and 4 days in Experiment 3). A secondary fall then occurs, which reaches its lowest level from 5 to 12 days after treatment, and this is followed by a secondary and, as far as has been determined, permanent rise, which persists for at least 54 days after x-ray treatment (Text-fig. 2). The slight variation in the time relations of the various phases of the curves, shown in Text-figs. 1, *a*, *b*, and 2 are probably due to the fact that in Experiments 1 and 3 the entire dose of x-rays was given in a single day, while in Experiment 2 the seven doses were dispersed over an interval of 8 days. A comparison of Text-figs. 1, *a* and 2 shows that the primary rise occurred on the 3rd and on the 9th day respectively, while the secondary fall reached its lowest limit on the 5th and on the 21st day. It seems possible that the destructive action of the x-rays on the lymphocytes is first felt by these cells in the general circulation and in the spleen (this corresponds to Warthin's view[5]), and that this accounts for the primary fall. In the meantime the lymphogenic cells of the other organs, lymph glands, bone marrow, etc., contribute cells to the blood, these being responsible for the secondary transient rise. When all the lymphogenic tissues have been affected by the x-rays the secondary fall in these cells is apparent in the blood, but it does not reach the extremely low level characteristic of the primary fall, because of beginning regeneration in the spleen. Regeneration of all the lymphogenic tissues later contributes to the permanent rise. It requires a considerable period of time before the lymphocyte-forming tissues regenerate entirely. In Experiment 2 for instance, the tissues are not contributing the normal numbers of lymphocytes to the circulation 54 days after the last x-ray treatment. The curve representing blood lymphocytes (Text-fig. 2) can be seen to be rapidly approaching the pre-treatment level at this time, however, and probably, had the observations been prolonged over a sufficiently long period of time, the lymphocytic elements would again have been present in the blood in normal numbers. When the x-ray treatments are distributed

over a number of days the issues are somewhat confused, and because of overlapping of phases the curves are not so sharply defined as when the entire dose is given in a single day. The primary fall in circulating lymphocytes, which in the case of the animals given x-rays in several doses is really the result of a series of primary falls, one following each treatment, is complete after 48 hours. However, the other phases, as may be seen from Text-fig. 2, are somewhat lengthened on this curve as contrasted with those given in Text-fig. 1, *a* and *b*.

From 24 to 48 hours after x-ray treatment there is, in most instances, a considerable increase in the polymorphonuclear neutrophilic leucocytes, which is well seen in the figures of Tables I and II. This is not evident in the rabbits followed (Tables VII, VIII, and IX) nor in the rats (Table VI). In mice (Table V) there is a tendency for these cells to exhibit a post-treatment rise, but this is not striking. The blood of the guinea pig showed a moderate increase in these cells after x-ray exposure.

When there is a primary rise in polymorphonuclear leucocytes, it is followed by a primary fall, which reaches its lowest level at a time when the lymphocytes are beginning to rise, the 6th day after x-ray exposure in Experiment 1, and the 3rd day in Experiment 2 (Tables I and II). Later there is a gradual rise to normal, about which level the number of neutrophilic cells fluctuates within wide limits. This is well seen in Table II. The polymorphonuclear cells are much less affected than the lymphocytes even when large doses of x-rays are given, and after the primary stimulation and later depression the return to the normal number per c. mm. occurs at a time when the lymphocytes are still at a very low level (Tables I and II). This seems to confirm Warthin's contention[5] that the bone marrow is affected somewhat later and to a more limited extent than the lymphogenic tissues proper. Circulating eosinophils and basophils, as well as large mononuclear and transitional leucocytes, usually share in the stimulations and depressions of the neutrophilic cells. This is what would be expected, as they all belong to the granular series and all originate in the bone marrow.

A glance at the curves shown in Text-figs. 3 to 10 leaves little to be said regarding the regularity with which the circulating lymphocytes of a series of animals of the same species respond to the same

dose of x-rays. Exceptions to the general rule of a quantitative relation between the reactions of the several animals of a series are uncommon. Apparently Rabbit 2 in Text-fig. 7 and Rabbits 1 and 2 in Text-fig. 9 belong in this category. The latter animals received the only treatment with a filtered dose of x-rays in the entire group reported, and it is not possible to draw conclusions from this single instance. The lymphocytic fall is evident, however, in both animals. Rabbit 2 in Text-fig. 7, then, is the only exception encountered and no explanation is apparent. There is a decrease in the circulating lymphocytes of this animal, following treatment with the x-rays, but this is not so great as in the companion rabbits, Nos. 1 and 3. Exceptions are naturally encountered in all biological experiments, and as this is the only striking instance found in the entire study it seems safe to disregard it and to accept the other results as manifesting accordance to a general law, inasmuch as they are regular and constant.

In Experiment 9 x-rays equivalent to 42 Holzknecht units destroyed, if all the rabbits are included, an average of 55.1 per cent, or, if Rabbit 2 is disregarded and the others (Nos. 1 and 3) with comparable lymphocyte curves are considered, an average of 69.4 per cent of the total number of circulating lymphocytes in the 48 hour interval following treatment. In the monkeys, given the same dose, Experiment 12, Table X, the effect of the x-rays was much more pronounced, 82.7 per cent of the circulating lymphocytes having disappeared 48 hours after treatment. It would seem that the monkey is more susceptible to the x-rays, in as far as the lymphocytic reaction represents an accurate measurement of the degree of susceptibility, than the rabbit. In the pony a dose of 160 Holzknecht units destroyed 95.4 per cent of the circulating lymphocytes, as determined 48 hours after treatment. In the monkey, Experiment 2, Text-fig. 2, Table II, 42 Holzknecht units were sufficient to destroy 93.1 per cent of the lymphocytes of the blood. This contrasts sharply with the three monkeys in Experiment 12, Text-fig. 10, Table X, in which the same dose destroyed an average of 82.7 per cent, and in the animal most affected, No. 3, but 84.7 per cent.

The lymphogenic tissues of the cat seem to be more susceptible than those of any other animal studied, inasmuch as 3 Holzknecht

units were sufficient to destroy 89.2 per cent of the circulating lymphocytes. In some of the experiments it was not possible to determine accurately the doses of x-rays given, but from the information at hand it seems that different species of animals vary considerably in their response to a given dose of x-rays. Those studied here would seem to follow a series, progressing from the most to the least susceptible, somewhat as follows: cat, monkey, guinea pig, rabbit, rat, mouse, and pony.

## SUMMARY.

1. X-rays in large doses affect the lymphocytes before any of the other circulating cells.

2. There is a sharp fall in the total number of circulating lymphocytes, which is complete 48 hours after x-ray treatment.

3. Following the immediate decrease in the circulating lymphocytes there is a primary rise, followed by another fall, which in turn is followed by a permanent rise of these cells to normal.

4. The effect of the x-rays on different species of animals varies considerably, but in those studied, cat, monkey, guinea pig, rabbit, rat, mouse, and pony, the selective action on the lymphocytes was in all instances apparent.

5. When several animals of the same species are given the same dose of x-rays, the effect on the circulating lymphocytes seems to be quantitatively parallel, when determined by blood counts.

6. The polymorphonuclear neutrophilic leucocytes, when affected at all, increase in number immediately after the administration of the x-rays and then tend to decrease below their normal level. This decrease is followed by a return to normal many days before the lymphocytes reach their original level.

7. The other cells of the blood follow the neutrophilic curve.

8. Percentage figures, as determined by differential blood counts, do not give an accurate indication of the effect of the x-rays. It is only when these are multiplied by the total white blood count that a figure, representing the total number of cells of the series per c. mm. of blood, is obtained, which varies to the stimulus in a constant manner, the variations being practically quantitative.

# STUDIES ON X-RAY EFFECTS.

## II. Stimulative Action on the Lymphocytes.*

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 27, 1918.)

Taylor, Witherbee, and Murphy[1] have reported on the destructive action of x-rays on the circulating lymphocytes, confirming and extending the earlier work on this subject. It was noted by Murphy in his studies on x-ray effects[2] that while large doses destroyed, a small dose of x-rays would bring about a stimulation of the lymphocytes. This observation was later applied experimentally.[3] In the earlier experiments the older type of x-ray tube was used, and it was practically impossible to establish a standard and uniform dose. With the introduction of the Coolidge tube the difficulty was eliminated to a large extent, and there was an opportunity to check this observation and extend it.

Mice have not been used here as in the previous experiments for the reason that blood counts could not be made on these animals more frequently than once a week without causing too marked a fluctuation.

### EXPERIMENTAL.

Brown rabbits of the same relative size were used in the nine experiments. All the animals were kept in separate cages. Several blood counts were made on these normal rabbits, and they were then exposed to the rays of a Coolidge tube. A dose of low penetration

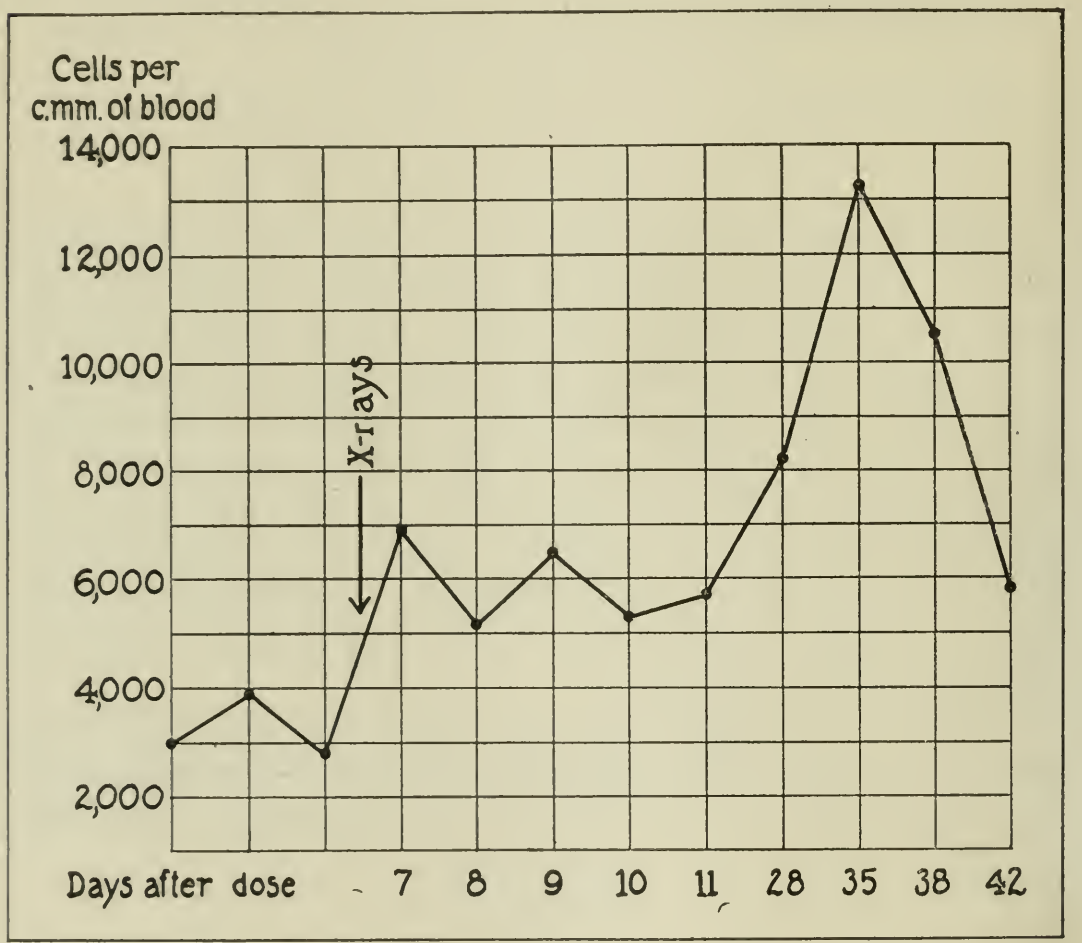
*This investigation was carried out by means of funds from the Rutherford Donation.

[1] Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, xxix, 53.

[2] Murphy, Jas. B., unpublished observation.

[3] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

was applied to the dorsal area: the spark-gap measured $\frac{7}{8}$ inch, the milliamperage was 25, the distance from the target to the back 8 inches, and the time of exposure 20 minutes. The temperature 8 inches from the target was 31°C. In almost every case a blood count made 48 hours after exposure showed a slight drop in the lymphocytes.

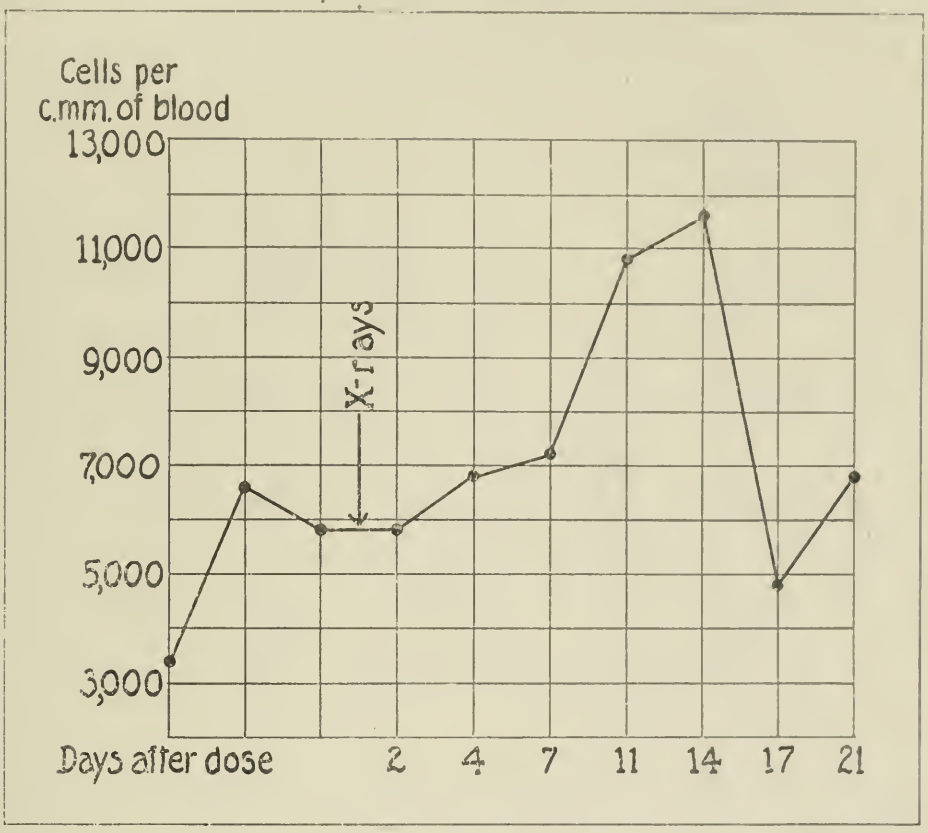



TEXT-FIG. 1. Effect of unfiltered x-rays of low penetration on the lymphocytes of a rabbit.

*Experiment 1* (*Text-Fig. 1*).—Three consecutive counts were made, the lymphocytes numbering 3,000, 4,000, and 2,800 respectively, and the rabbit was then exposed to x-rays. The count made 1 week later showed a stimulation in the cells, the number of lymphocytes being 6,900. Counts made at intervals showed slight fluctuation until the 28th day, when the count reached 8,300. On the 35th day there were 13,300 absolute lymphocytes. In the course of the 7 days following the last count the number of cells fell to 5,800.

*Experiment 2* (*Text-Fig. 2*).—Considerable fluctuation was shown in the normal counts. The first count 48 hours after exposure to x-rays showed little or no change, and for 7 days afterwards there was little increase, the lymphocytes numbering 5,000, 6,000, and 7,000 respectively, but on the 11th day the number had risen to 10,500, and on the 14th day to 11,600. They fell to 4,800 on the 17th day and in the last count rose to 6,900.

*Experiment 3*.—The standardizing counts were 6,900, 6,370, and 7,000. 48 hours after exposure to the x-rays a slight fall was noted, the number of cells



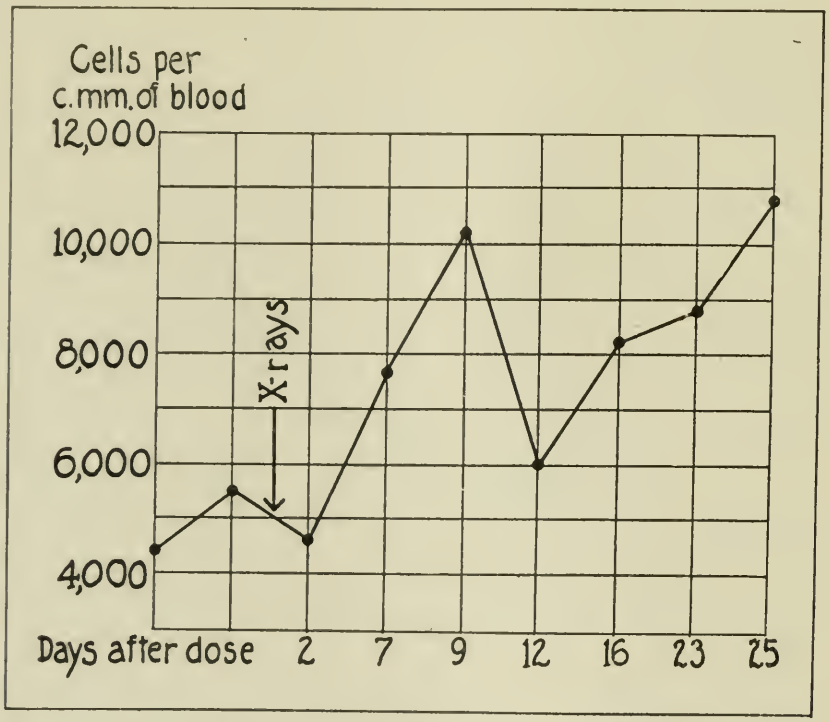

TEXT-FIG. 2. Effect of unfiltered x-rays of low penetration on the lymphocytes of a rabbit.

at that time being 5,100. On the 4th day there were 5,300 lymphocytes, and 6 days afterwards 10,240, showing marked stimulation. On the 8th day the number had decreased to 6,100 and on the 15th day to 4,000. After that there was a slight rise (6,500) on the 18th day and also on the 23rd, followed by another fall to 3,410. A rise to 8,200 was noted on the 64th day, and the last count, at which the number of cells was 6,300, was made on the 70th day after exposure.

*Experiment 4*.—4,300, 4,670, and 4,900 were the lymphocyte counts of a rabbit before x-ray treatment. 48 hours after exposure there was a slight increase (6,600). The 4th day the number of cells was 4,500, the 6th day 6,500, and the

15th day, when the last count was made, 5,979. This rabbit did not show a stimulation as did those of the preceding experiments.

*Experiment 5* (*Text-Fig. 3*).—At the two counts preceding x-ray treatment there were 4,560 and 5,300 lymphocytes respectively. 48 hours after exposure to the x-rays there was a slight fall to 4,600, and on the 7th day a rise (7,600) which continued to the 9th day and at that time reached 10,200. On the 12th day there was a slight decrease, but from that time to the 25th day, on which the last count was made, there was a continued rise in the number of lymphocytes, which finally reached 10,900.



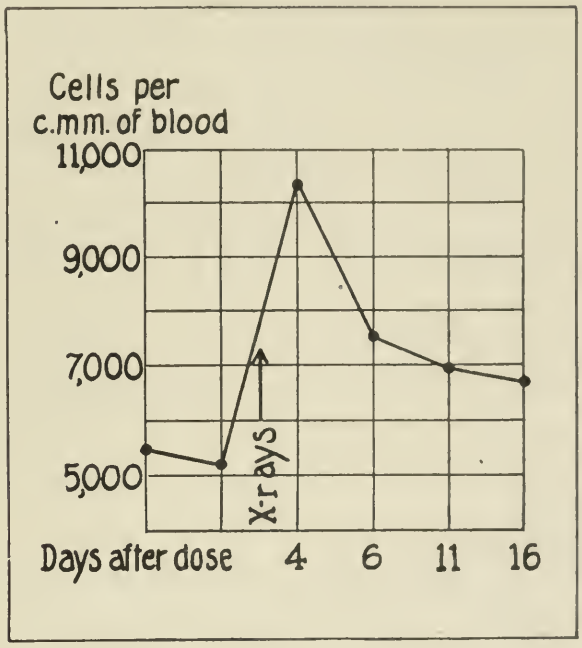

TEXT-FIG. 3. Effect of unfiltered x-rays of low penetration on the lymphocytes of a rabbit.

*Experiment 6.*—The normal counts showed a great fluctuation, the lymphocytes numbering 3,500 at the first count, at the second 6,100, and at the third 6,290. 48 hours after x-ray exposure they fell to 3,600, rose on the 4th day to 6,400, and fell again on the 6th day to 3,800. The cell counts, which were made at intervals until the 25th day after exposure, continued to rise and fall, never reaching a point beyond 5,900 or below 3,000.

*Experiment 7.*—The normal counts in this instance were 4,900 and 5,800. 48 hours after exposure to the x-rays the cells numbered 5,600, the 4th day 5,400, the 6th 5,700, and the 8th 6,180, showing a slight increase. On the 13th day the number of cells was 5,600, and on the 20th day, when the last count was made, 5,750.

*Experiment 8.*—2,270, 5,340, and 2,267 were the three consecutive lymphocyte counts made before x-ray treatment on a rabbit. 48 hours after treatment there was little or no change, the cells numbering 2,200. On the 4th day there was a rise to 4,800, on the 6th to 7,900. 11 days after exposure the number was 6,560. Subsequently there was a decline to 3,900 (13th day) and finally, on the 25th day, to 3,000.

*Experiment 9* (*Text-Fig. 4*).—Two normal counts, the first 5,500, the second 5,100, were followed by the short x-ray dose. The 48 hour count was not made on this rabbit. The first count following exposure was made 4 days afterwards, the lymphocytes then numbering 10,390. On the 6th day there was a fall to 7,500, on the 11th day to 6,900, and on the 16th day to 6,700.

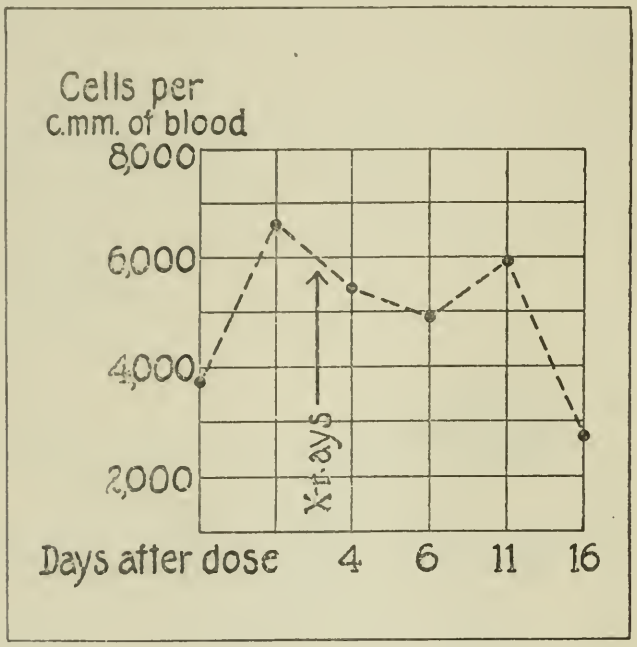



TEXT-FIG. 4. Effect of unfiltered x-rays of low penetration on the lymphocytes of a rabbit.

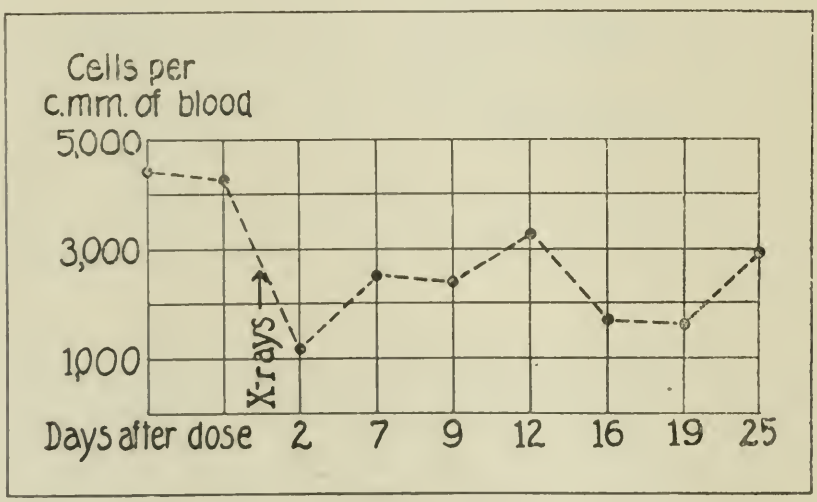
A comparable dose of filtered x-rays[4] was used also on a smaller number of brown rabbits (spark-gap 6 inches, milliamperage 5, distance from the target to the back 10 inches, time 26 minutes and 57 seconds). The rays were filtered through 3 mm. of aluminum. The animals were exposed in the same way (over the dorsal area) and kept under the same conditions as those of the preceding experiments.

[4] Remer, J., and Witherbee, W. D., *Am. J. Roentgen.*, 1917, iv, 303.

*Experiment 1, a* (*Text-Fig. 5*).—In the two counts preceding x-ray exposure the number of lymphocytes was not very constant, being 3,700 at the first and 6,600 at the second. After the filtered x-ray dose (6 inch spark-gap, milliamper-




TEXT-FIG 5. Effect of filtered x-rays on the lymphocytes of a rabbit.




TEXT-FIG. 6. Effect of filtered x-rays on the lymphocytes of a rabbit.

age 5, distance from the target to the back 10 inches, and time 26 minutes and 57 seconds) the count showed little change, 5,470 in the first count, which was made on the 4th day, 4,900 on the 6th day, 5,900 on the 11th day, and 2,733 on the 16th day.

*Experiment 2, a* (*Text-Fig. 6*).—The lymphocytes numbered 4,400 and 4,350 at the two counts preceding x-ray treatment. 48 hours after exposure there was a marked fall (1,230), and on the 7th day a slight rise (2,500). On the 9th day the count was similar to that of the 7th; on the 12th it was 3,500, on the 16th 1,700, on the 19th 1,720, and on the 25th 2,981. There is no sign of stimulation in Experiment 2, *a*.

## DISCUSSION.

It is of interest to note in these experiments that the x-ray dose used was of low penetration, the spark-gap being under an inch. The use of a larger spark-gap with apparently the same dose of x-rays did not give a stimulation. This suggests that the effect on the lymphoid organs is not the result of a direct action of the rays but is secondary to changes brought about either in the circulating blood or in the superficial tissues. The amount of x-rays penetrating to the deeper structures with this dose must be infinitesimal.

Another question arises as to the nature of the energy generated by the x-ray tube operated on so small a spark-gap. This point has not yet been taken up, but it is conceivable that other factors than the pure x-rays may play a part.

The results obtained in this small series of animals would not in themselves be accepted as conclusive evidence but are of interest principally as a parallel to our histological studies.[5] It is conceivable that a marked stimulation may be taking place in the lymphoid organs without a proportionate number of these cells being thrown into the circulation. The question in itself offers an interesting problem of just what determines the number of cells in the circulation. It is well known that individuals with normal counts react differently in the number of cells thrown into the circulation in response to infections. So here, even with marked stimulation taking place in the lymphoid tissue of the glands and spleen, in only a part of the animals perhaps could we expect this stimulation to be evidenced by an increase in the number of lymphocytes in the circulating blood.

[5] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 83.

SUMMARY.

This study consists of blood counts on nine rabbits after an exposure to x-rays of a $\frac{7}{8}$ inch spark-gap, milliamperage 25, distance from the target 8 inches, and time of exposure 20 minutes.

In seven of the nine animals there resulted an increase of the circulating lymphocytes. In five of these the increase was marked and in two others definite but not striking.

Of the two animals which showed no stimulation one showed marked fluctuation of counts both before and after x-rays and the other little or no change.

The higher penetrating dose (6 inch spark-gap, milliamperage 5, distance from the target 10 inches, time 26 minutes and 57 seconds) given to two animals produced no appreciable stimulation.

# STUDIES ON X-RAY EFFECTS.

## III. Changes in the Lymphoid Organs after Small Doses of X-rays.

By WARO NAKAHARA, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 8 and 9.

(Received for publication, October 21, 1918.)

The observations of previous investigators have emphasized the fact that x-rays are preeminently and selectively destructive to lymphoid cells (Heineke[1]). Thomas, Taylor, and Witherbee[2] have shown that small doses of x-rays will bring about a stimulation of the circulating lymphocytes, confirming the earlier observations made in this laboratory.[3] As in the experiments on the effect of heat on the circulating lymphocytes,[4] there was a fall immediately after exposure, followed by a more or less gradual rise.

The histologic findings in the lymphoid tissues of mice treated with heat have already been reported,[5] and it has been shown that the lymphocytosis induced by heat is due to the proliferation of certain cells in reaction to the extensive destruction of the tissue by this agent. *A priori*, since x-rays are known to be destructive to lymphoid tissue, the lymphocytic changes observed seemed to be due to a similar cause. The experiments to be reported here were undertaken in order to determine this point.

[1] Heineke, H., *Münch. med. Woch.*, 1903, l, 2258; *ibid.*, 1904, li, 1382; *Mitteil. Grenzgeb. Med. u. Chir.*, 1905, xiv, 21.

[2] Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.

[3] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[4] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.

[5] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17.

## EXPERIMENTAL.

Six normal rabbits of the same color (white), and of approximately the same age, were treated with the same dose of x-rays as was used by Thomas, Taylor, and Witherbee (spark-gap $\frac{7}{8}$ inch, milliamperage 25, distance 8 inches, time 20 minutes). The animals were killed at intervals, and tissues were taken (*a*) immediately, (*b*) 48 hours, (*c*) 4 days, (*d*) 7 days, (*e*) 10 days, and (*f*) 14 days after treatment. This experiment has been duplicated with six brown rabbits. In the second experiment, however, animals were killed two by two (*a*) 24 hours, (*b*) 3 days, and (*c*) 14 days after they were x-rayed, these periods being found to be critical from the result of the first experiment.

For fixation, Carnoy's chloroform-alcohol-acetic (6:3:1) was easy to use and gave good results. Its use saved much time by dispensing with the necessity of passage through the grades of alcohol. Some tissues were fixed in Zenker's fluid for control. The staining was done almost exclusively with Heidenhain's iron-hematoxylin, though a few sections were stained by the ordinary method of hematoxylin and eosin.

### *Results.*

*Spleen.*—The general histologic condition of the spleen immediately to 24 hours after the treatment is approximately normal. Necrotic cells are extremely scarce in both the nodule and the pulp. The number of mitotic figures in the germinal center of the nodule varies; as a rule, it shows in section a few, sometimes several, and more rarely ten. In the normal spleen of the adult rabbit the germinal centers contain but few mitotic figures, and frequently none. The excessive abundance of mitotic figures after treatment may therefore be taken to indicate the stimulation of these cells.

Sections taken 48 hours after treatment show a condition apparently identical to that just described. The number of necrotic cells in the pulp seems to be slightly larger if anything, but not large enough to be regarded as abnormal.

4 days after the treatment the signs of stimulation become most marked. While the general histologic condition remains unchanged,

the number of mitotic figures in the germinal center of the nodule is decidedly increased. The germinal center is seen in the section to contain usually several, but sometimes about ten, mitotic figures (Figs. 1 and 2). A comparison of this with the normal condition, in which the proliferative activity in the germinal center is limited, seems to warrant the conclusion that the small dose of x-rays acts as a stimulant to the proliferation of the lymphoid cells.

Sections were taken on the 7th, 10th, and 14th days after treatment. The general histologic condition of all the sections was found to be normal, with the exception of a slightly enhanced mitosis. In the sections taken on the 7th day the number of mitotic figures in the germinal center of the nodule was seen to be smaller, and the tendency to decrease was manifested even more markedly in the sections taken later (on the 10th or 14th day). Even in the later sections, however, the proliferative activity of the cells, judged from the standpoint of mitosis, seemed slightly above the normal.

*Lymph Glands.*—In addition to the mesenteric lymph gland, cervical, axillary, and inguinal glands were taken for comparison. The changes in these glands were found to be so nearly uniform in character that a general description will apply to all of them.

The most striking deviation from the normal in the histology of lymph glands immediately after the treatment is the great abundance of mitotic figures in the nodule. The dividing cells are not localized, but are found all over the nodule. Some mitotic figures are seen in the internodular spaces in the cortex and also in the medulla, where, as a rule, very few if any division figures are normally present. The whole tissue was seen to be almost entirely free from degenerating cells. Some evidences of necrosis were, of course, observed here and there, but they were no more conspicuous than those seen in normal tissue.

48 hours after treatment the signs of stimulation of the cells were even more evident than immediately after treatment. All nodules contained excessively large numbers of dividing cells, each of the ordinary sized nodules showing in a section at least ten, and not infrequently as many as twenty mitotic figures (Figs. 3 and 4). The general condition of the gland is similar to that immediately after the treatment, except that there is a slight increase of necrotic cells, mainly in the pulp spaces.

Mitotic figures appear slightly less numerous in the tissue taken 4 days after treatment. The nodule usually shows several mitotic figures in a section. These conditions may still be taken as slightly abnormal. The occurrence of the dividing cells is more or less localized in the nodule, and in many instances they constitute rather typical germinal centers.

In sections taken 7, 10, and 14 days after the treatment, conditions are similar to those just described. The slightly excessive occurrence of mitosis as late as the 14th day is well shown in many nodules in the form of actively proliferating cells of the germinal center.

## DISCUSSION.

As far as the results of lymphocyte counts show, the nature of lymphocytosis induced by heat and of that brought about by x-rays is indistinguishable, since the lymphocytic changes are exactly parallel in both cases; *i.e.*, always with a characteristic fall preceding the marked rise. The idea that the phenomenon of lymphocytosis is of the same nature, regardless of whether the agent used for its production is heat or x-rays, seems probable, especially when we recall the results of Heineke[1] and Warthin,[6] who have shown that the effect of x-rays on lymphoid tissue is, in the main, similar to that of heat, as described by us in another paper.[5] They used the x-rays, however, in different dosage from ours, and did not, furthermore, make observations during the critical period after the x-ray treatment, when an excessive multiplication of the cells may possibly take place. Notwithstanding the apparent similarity in the nature of the two phenomena which have been pointed out, the results of the experiments described in the present paper show conclusively that the lymphocytosis induced by the small dose of x-rays is due to the primary stimulative effect of the agent and hence is fundamentally different in nature from the similar lymphocytic change induced by heat, which is a sort of regenerative phenomenon.

Throughout the course of the experiment no indication has been observed that suggests the injurious effect of the dose upon any of the

[6] Warthin, A. S., *Physician and Surg.*, 1907, xxix, 1.

lymphoid tissues examined. On the other hand, mitotic figures were seen to become gradually more abundant after the treatment. In the spleen this enhanced proliferative activity of the cell reached its height about 4 days after the treatment, and the more or less distinct indications of the stimulation persisted in the germinal center up to the 14th day after the treatment. In the lymph glands the stimulative change is distinctly manifested earlier and is more extensive than in the spleen.

These histologic findings are in harmony with the results of the blood cell counts, which show that the increase of lymphocytes becomes most pronounced about 1 week after the treatment. If the lymphocytosis is due to the stimulation of the lymphoid tissues, the latter should show the change before the former becomes evident, and this is apparently what takes place.

## SUMMARY AND CONCLUSION.

1. The small dose of x-rays applied to the rabbit has no appreciable destructive effect on the lymphoid tissue.

2. Indications of stimulation of the lymphoid tissue appear immediately after the treatment, become most pronounced in 2 (in lymph glands) to 4 (in the spleen) days, and persist, in a slight degree, up to the 14th day.

3. These facts suggest that the lymphocytosis induced by the small dose of x-rays is due to a primary stimulative effect upon the lymphoid tissue of the animal.

## EXPLANATION OF PLATES.

### PLATE 8.

FIG. 1. A splenic nodule, 4 days after the treatment, showing extensive stimulation. × 350.

FIG. 2. The same, showing mitotic figures (*M*) in higher magnification. × 1,000.

### PLATE 9.

FIG. 3. A nodule of the mesenteric lymph gland, 48 hours after the treatment, showing intense stimulation. × 350.

FIG. 4. The same, showing mitotic figures (*M*) in higher magnification. × 1,000.

# STUDIES ON X-RAY EFFECTS.

## IV. Direct Action of X-rays on Transplantable Cancers of Mice.*

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(Received for publication, October 21, 1918.)

It has been shown in this laboratory that x-rays administered in doses sufficient to destroy a large proportion of the lymphoid tissue reduce the resistance of the animal to transplanted cancer.[1] Furthermore, both potential and established induced immunity can be destroyed by a similar process.[2] It has also been shown that small doses of x-rays sufficient to stimulate the lymphocytes increase the resistance to cancer.[3] These observations bring up a number of interesting points with regard to x-rays as a therapeutic agent in the treatment of cancer. The literature on this subject is so extensive and contradictory that no attempt will be made to review the previous work. The question of immediate interest to us in this investigation is whether or not x-rays, even in a dose above that possible for therapeutic purposes, will kill the cancer cell. It is necessary in the light of the experiments mentioned above to rule out the action of this agent on the animal itself.

To determine the cumulative, as well as the immediate effect of the direct action of powerful doses of x-rays upon tumor cells, we undertook to grow a Bashford mouse tumor (Adenocarcinoma No. 63) in successive generations of white mice, subjecting the tumor to like doses of x-rays after excision and before inoculation at each transplantation. An actively growing tumor which had been propagated

*This investigation was carried out by means of funds from the Rutherford Donation.

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[2] Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1.

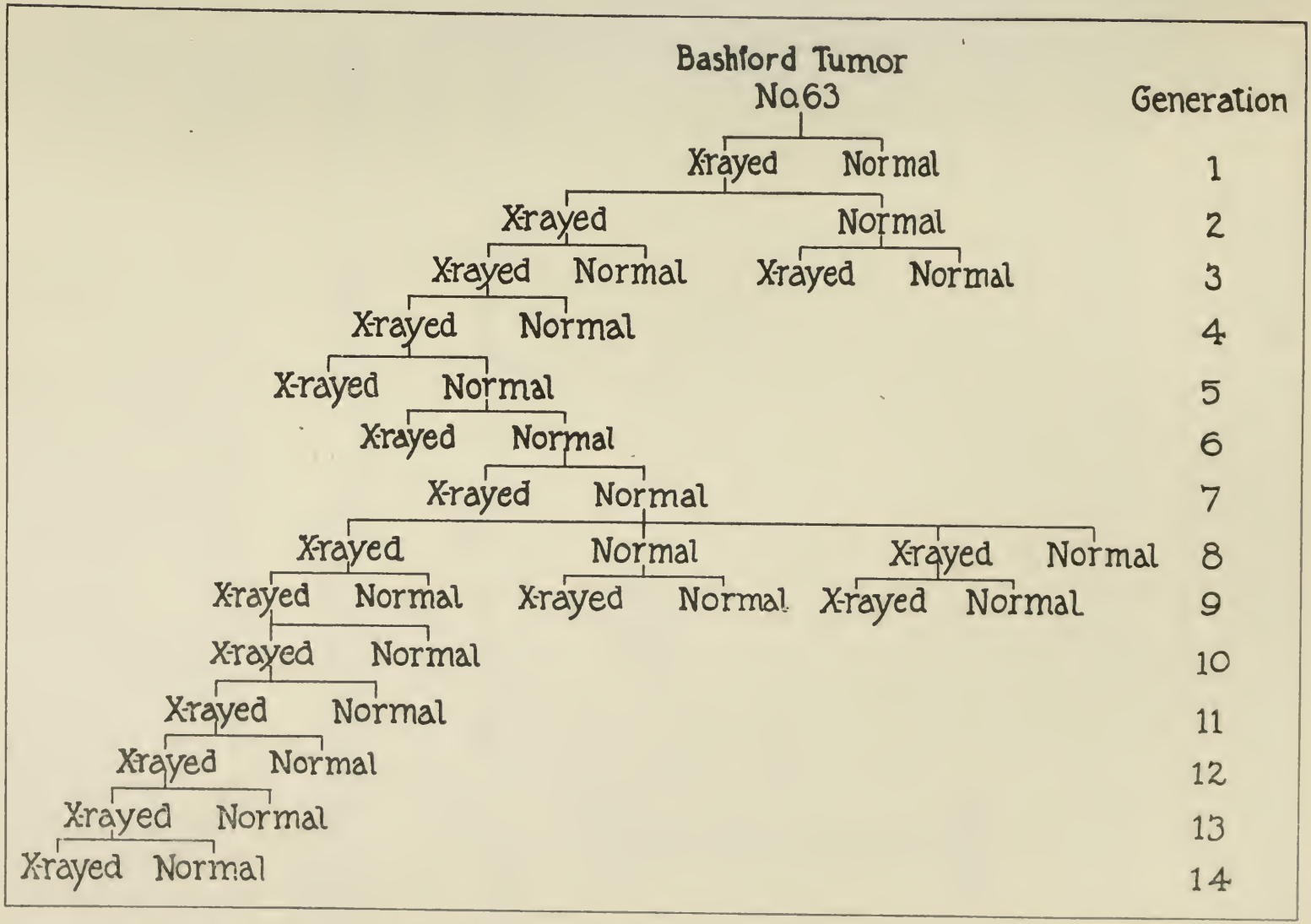
[3] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

in the laboratory for some time was used, and an x-ray dose selected which was purposely above the range of therapeutic dosage: Coolidge tube, spark-gap 8 inches, milliamperes 5, distance 6 inches, and time 2 minutes and 35 seconds. The percentage of takes and rate of growth of the tumor were observed in fourteen generations, extending over a period of 17 months with eleven exposures between transplantations.

Later, considering the possibility of greater absorption of less penetrating rays, we undertook a similar experiment with the following dose: Coolidge tube, spark-gap 1 inch, milliamperes 25, distance 8 inches, and time 20 minutes. In this experiment the tumor was observed in four generations.

*Method.*

In both experiments (Nos. 1 and 2), the technique used was as follows: Wherever possible a healthy, actively growing tumor, approximately 1.5 by 1.5 cm., was chosen for inoculation. The mouse was killed and the tumor removed under aseptic conditions. It was then cut into halves and each half placed in a small Petri dish, covered with a single layer of sterile gauze, and set into a large Petri dish containing sufficient salt solution to keep the gauze slightly moist. One-half remained in the laboratory at room temperature, while the other half was subjected to x-rays. The temperature at the surface of the gauze did not rise above 33°C. in Experiment 1, or 42°C. in Experiment 2. The x-rayed portion of the tumor was then divided into small pieces of uniform size. Care was taken to select for inoculation the outer actively growing portions of the tumor. Asepsis was observed throughout the experiment. Single pieces of the tumor were loaded into hollow needles and ten normal white mice (young adults) were inoculated in the right groin. In loading the needles care was taken to macerate the tissue as little as possible. The control half of the tumor which received no x-rays was then inoculated into ten mice in exactly the same manner. In Experiment 1 the x-rayed half remained out of the body 45 to 50 minutes and the control half 1 hour. In Experiment 2 the x-rayed half remained out of the body 60 to 70 minutes and the control half $1\frac{1}{4}$ hours. The tumors resulting from the inoculations were measured at periods of 1 week




TEXT-FIG. 1. The generations of tumor transplantations for Series 1, extending over a period of 17 months. The final tumor transplant had been exposed to eleven treatments of x-rays during this period.

until the death of the mice. A healthy tumor was selected from the x-rayed series unless because of an epidemic or some other cause this was impossible, when one of the control tumors was chosen for treatment and an inoculation was made into another series of twenty mice in the manner described.

The first series of transplants was begun December 11, 1916, and terminated May 9, 1918 (Text-fig. 1). In Generations 5 and 6 the

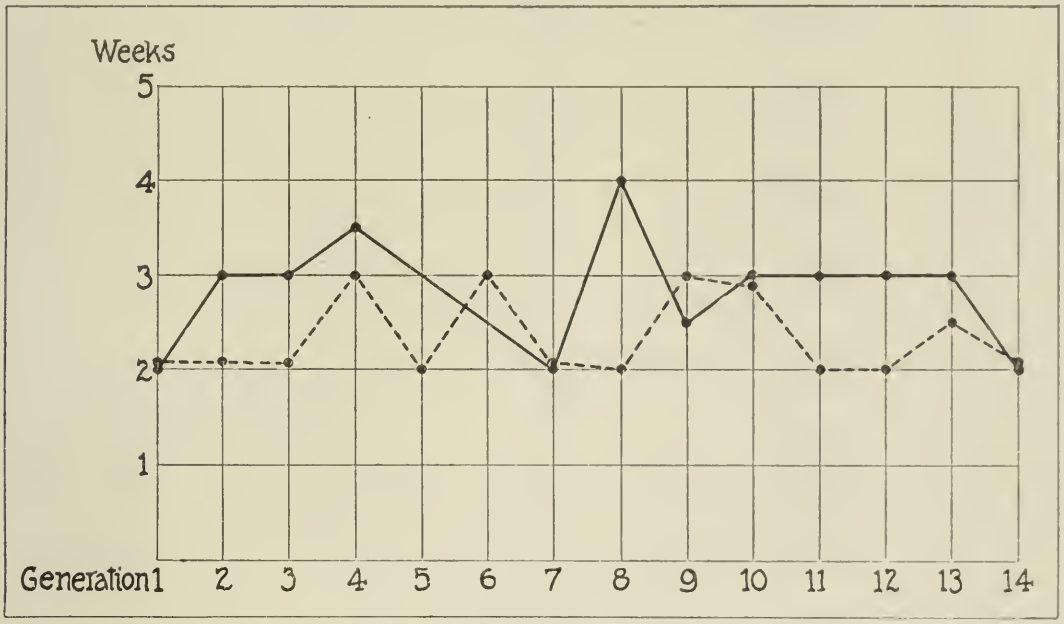



TEXT-FIG. 2. A comparison of the number of takes between x-rayed and untreated cancer on inoculation into mice. .....Controls. ————X-rayed cancers.

percentage of takes is inaccurate, probably much too low, on account of an epidemic among the mice which occurred before the usual time of appearance of tumors. For the transplants at this period, on account of the loss from the epidemic, the tumors of the next generation were taken from the control series. If we disregard the sudden drop in those two generations, the percentage of takes among the mice receiving x-rayed portions of the tumor remained between 25 and 45 during the first year, or nine generations. At the same time the percentage of takes among the control mice was between 50 and 70.

From the tenth generation to the fourteenth the percentage of takes does not remain consistently higher among controls and in both x-rayed and normal groups the percentage rises even to 100 (Text-fig. 2).

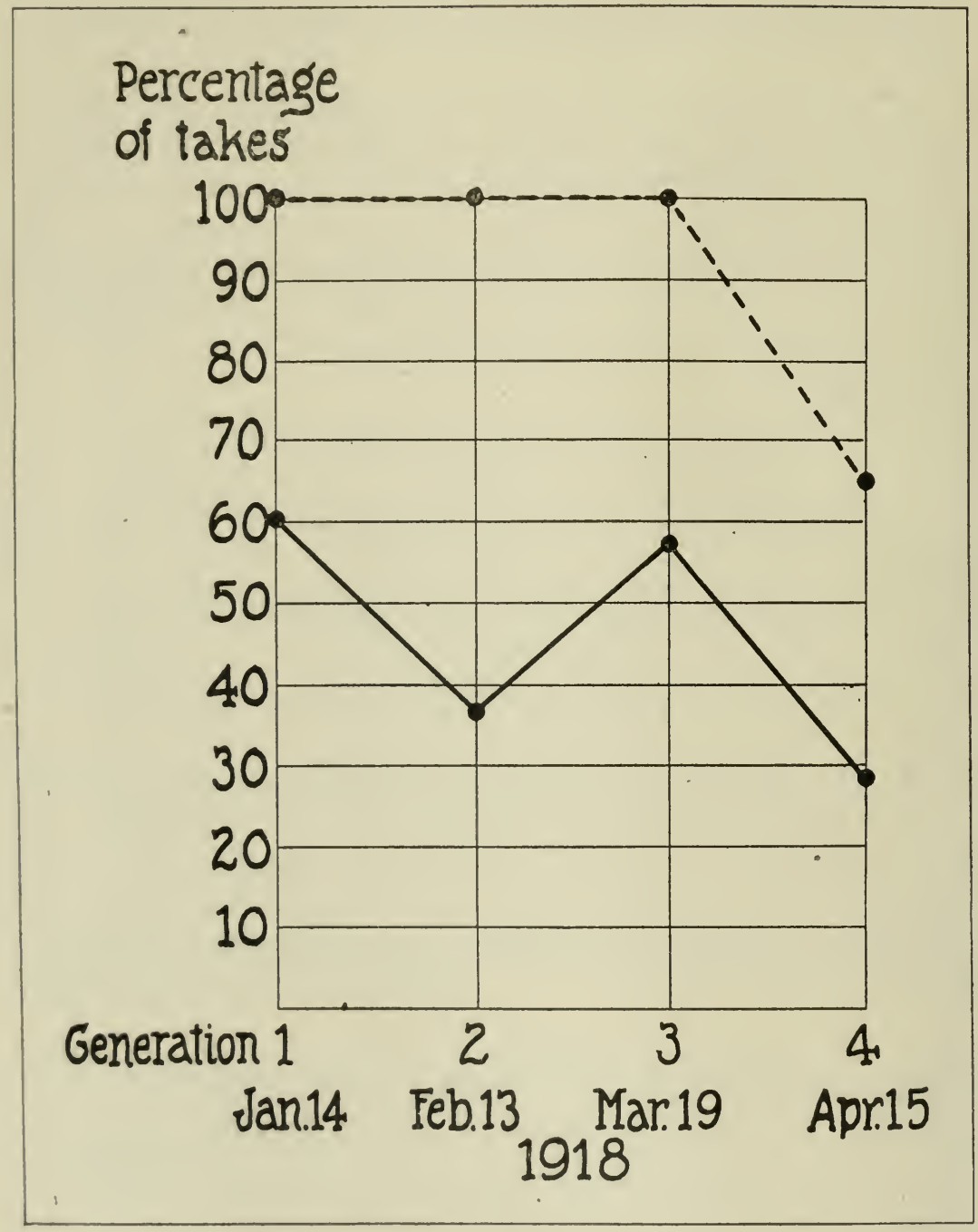
The rate of growth of the tumors was judged from curves representing the average time of appearance of the tumors and the average size 5 weeks after transplanting. As these two curves were essentially similar, only the former is illustrated (Text-fig. 3). In less than half the generation groups the tumors appeared a week earlier in the control mice than in those which had received the x-rayed



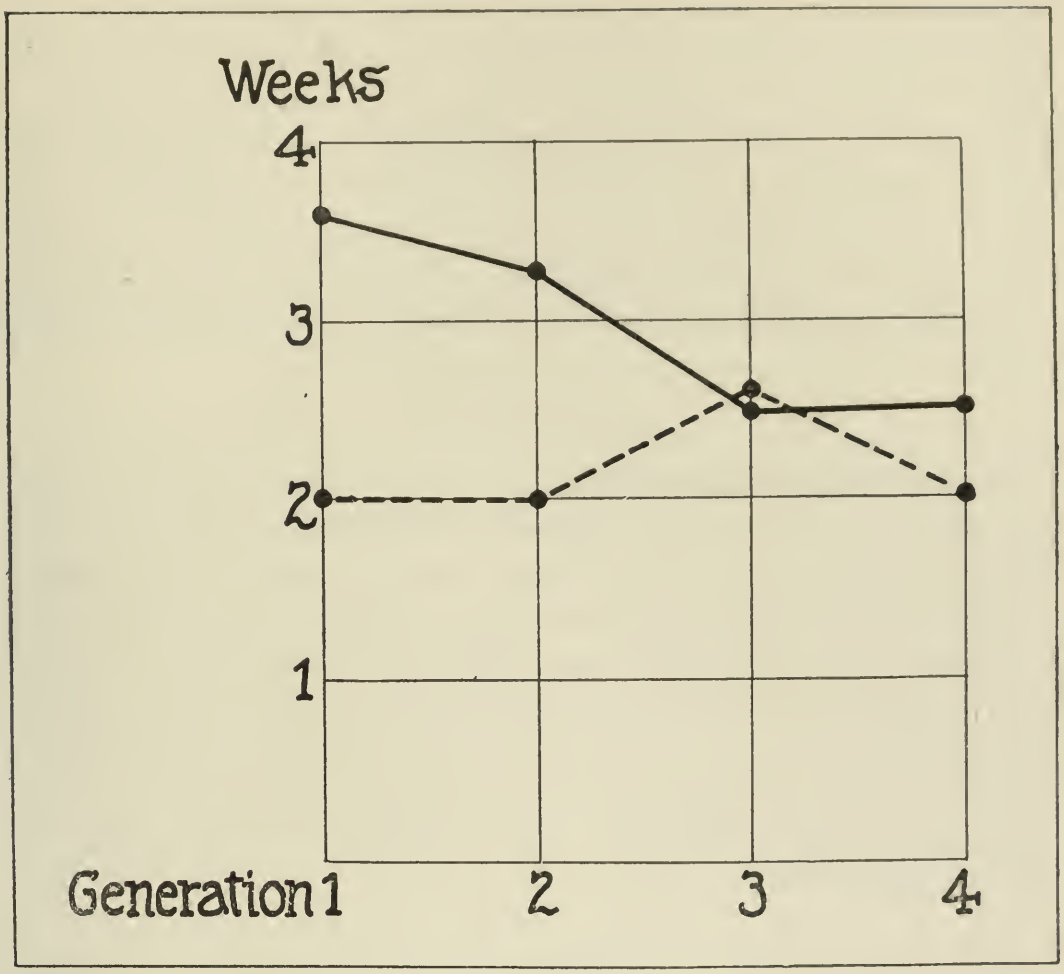

TEXT-FIG. 3. A comparison of the average time of appearance of tumors with and without exposure to x-rays. .....Controls. ————X-rayed cancers.

tumor. The difference in size between the tumors of the two groups 5 weeks after transplantation is slight and not consistent.

Experiment 2 was begun January 14, 1918, and terminated May 9, 1918. Four successive generations were observed. Without a much longer series of transplants it is impossible to judge the cumulative effect of this dose of x-rays on the general virulence of the tumor. The difference in the percentage of takes is consistently much more marked in this series than at any point in the first series (Text-fig. 4). Initially the rate of growth of the tumors receiving x-rays was also greatly retarded (Text-fig. 5).




TEXT-FIG. 4. A comparison of the percentage of takes for Series 2. .....Controls. ———X-rayed cancers.




TEXT-FIG. 5. Average time of appearance of tumors, x-rayed and untreated, in Series 2. .....Controls. ————X-rayed cancers.

## DISCUSSION.

The present tendency of workers on x-ray therapy of cancer is to devise methods of increasing the amount of x-rays delivered at the location of the cancer process. In the light of our observations there is one point which should be taken into consideration; that is, whether or not we are justified in using a procedure which apparently only inhibits the cancer temporarily, while it incidentally lowers the resistance of the individual to the growth. It is well recognized that a proportion of cancers held in check for a time by x-ray treatment will later grow more rapidly. It is not possible to form an idea of what proportion of the total number of patients treated show this result, as few completely and accurately controlled series have been

published. Blood counts on a number of these individuals have been made in this laboratory, and they all showed remarkably low lymphoid counts. Our work, however, has been done mainly on cancer of mice, and we are therefore not warranted in drawing sweeping conclusions until there is more careful confirmation from studies on man. We feel justified, however, in suggesting that powerful doses of x-rays which are only capable of inhibiting cancer growth for a time may bring about eventually a lowered resistance to a return of the disease process.

## CONCLUSIONS.

These experiments indicate that the direct action of x-rays in more powerful doses than can be applied therapeutically is somewhat injurious to tumor cells, but by no means destroys them. Experiment 1 also indicates that the cancer cells establish a resistance to the x-rays after repeated doses. This harmonizes with the experience of clinicians who have succeeded in checking cancerous growths for some time but reach a point where no response can be effected by repeated doses. The rays of low penetration used in Experiment 2 are apparently more harmful to tumor cells than the penetrating rays used in Experiment 1.

# BLOOD COUNTS IN EXPERIMENTAL POLIOMYELITIS IN THE MONKEY.

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(Received for publication, October 30, 1918.)

When poliomyelitic virus has once been established in the monkey, it becomes more virulent for this animal by repeated passage.[1] Hence the virus used in experimental work usually brings about a severe form of the disease and high death rate. Occasionally an animal survives the stage of complete prostration if carefully attended and recovers with residual contractures. Experimental poliomyelitis in the monkey, then, is comparable with the severer forms in man, and observations on the variation in leucocyte count in the circulating blood would therefore be expected to yield more nearly uniform results than studies on human cases. In the latter there are many factors of difference in reaction and resistance, age and virulence of the virus, which might contribute to variation. Moreover, opportunities for observations during the incubation period in human cases are rare.

It is generally accepted that abnormal white blood counts are constantly found in poliomyelitis, but opinion is divided on the characteristics of this change.

Müller[2] asserts that a distinct leucopenia with a relative lymphocytosis is characteristic and pathognomonic of the febrile stage, while La Fétra[3] had previously described a moderate leucocytosis as being characteristic of the acute stage in human beings.

Gay and Lucas[4] summarize their blood findings as follows: "The acute stage of anterior poliomyelitis, as it occurs in human beings, and as it is produced experimentally in monkeys, is characterized by the occurrence of a distinct leuko-

[1] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 195.

[2] Müller, E., Die spinale Kinderlähmung, Berlin, 1910.

[3] La Fétra, L. E., *Arch. Pediat.*, 1909, xxvi, 328.

[4] Gay, F. P., and Lucas, W. P., *Arch. Int. Med.*, 1910, vi, 330.

penia. The differential count shows a relative increase in number of eosinophils and lymphocytes."

Peabody, Draper, and Dochez[5] found in human cases a constant and marked leucocytosis. They also found a constant increase in polymorphonuclear cells of 10 to 15 per cent and a diminution of lymphocytes of 15 to 20 per cent.

## OBSERVATIONS.

Blood counts were made on six series of monkeys as follows: Series I, 40 normal monkeys for comparison (Table I); Series II, 4 monkeys already prostrate from experimental poliomyelitis (Table II); Series III, 12 monkeys during the incubation period, later developing the disease; Series IV, 5 monkeys which received the virus but did not develop the disease; Series V, 4 monkeys during the period of recovery from the acute stage; Series VI, 5 monkeys which had passed through the attack and recovered with residual paralyses.

In order to reduce the hourly variations in the blood counts the samples were collected at about the same time on the days of observation between 10.30 and 11.30 a.m.

*Series I.*—121 counts were made on 40 healthy, adult monkeys (*Macacus rhesus*). Averages of all counts are recorded in Table I.

TABLE I.

*Average of 121 White and Differential Blood Counts on 40 Normal Monkeys.*

| White cells per c.mm. | Lymphocytes. | | Polymorphonuclear leucocytes. | | | Large mononuclear and transitional leucocytes. | Total lymphocytes. | | Total polymorphonuclear neutrophilic leucocytes per c.mm. |
|---|---|---|---|---|---|---|---|---|---|
| | Small. | Large. | Neutrophilic. | Eosinophilic. | Basophilic. | | | | |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | | |
| 22,181 | 47.5 | 6.2 | 41.1 | 4.1 | 0.4 | 0.6 | 53.7 | 11,815 | 9,116 |

*Series II.*—Seven counts on four monkeys paralyzed as a result of experimental inoculation with the virus of poliomyelitis are recorded in Table II.

[5] Peabody, F. W., Draper, G., and Dochez, A. R., A clinical study of acute poliomyelitis, Monograph of The Rockefeller Institute for Medical Research, No. 4, New York, 1912, 97.

TABLE II.

*White and Differential Blood Counts on Monkeys Prostrate after Poliomyelitic Infection.*

| Monkey No. | Length of time after inoculation. | Day of prostration. | White cells per c.mm. | Lymphocytes. | | Polymorphonuclear leucocytes. | | | Large mononuclear and transitional leucocytes. | Total lymphocytes. | | Total polymorphonuclear neutrophilic leucocytes per c.mm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Small. | Large. | Neutrophilic. | Eosinophilic. | Basophilic. | | | | |
| | *days* | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | | |
| 1 | 17 | 2 | 8,875 | 13.7 | 6.3 | 74.7 | 2.0 | 0.0 | 3.3 | 20.0 | 1,775 | 6,630 |
| 2 | 37 | * | 9,150 | 20.0 | 3.3 | 76.0 | 0.3 | 0.0 | 0.3 | 23.3 | 2,132 | 6,954 |
| | 38 | 1 | 8,275 | 24.3 | 2.7 | 70.0 | 3.0 | 0.0 | 0.0 | 27.0 | 2,234 | 5,793 |
| | 41 | 4 | 4,900 | 21.6 | 3.3 | 73.7 | 0.7 | 0.0 | 0.7 | 25.0 | 1,225 | 3,611 |
| | 47 | 10 | 4,675 | 28.3 | 18.7 | 53.0 | 0.0 | 0.0 | 0.0 | 47.0 | 2,197 | 2,478 |
| 3 | 10 | 2 | 17,550 | 15.7 | 1.7 | 82.7 | 0.0 | 0.0 | 0.0 | 17.4 | 3,054 | 14,514 |
| 4 | 10 | 3 | 21,600 | 14.0 | 5.7 | 80.3 | 0.0 | 0.0 | 0.0 | 19.7 | 4,255 | 17,345 |

* Almost prostrate; both legs flaccid; left arm and back weak.

Monkey 1 of this series received the virus by the nasal route. An intraspinal injection of 2 cc. of normal horse serum had been given on the day preceding the application of the virus to the nasal mucous membrane. After 10 days the monkey became excitable, after 11 days ataxic, and on the 15th day it became prostrate. The blood count was made on the 17th day after infection, which was the 8th day of the disease and the 2nd day after the monkey had become completely prostrated. Death occurred on the 21st day, and autopsy showed well defined lesions of poliomyelitis.

Monkey 2 was inoculated intracerebrally with an incubated mixture of 0.2 cc. of a Berkefeld filtrate of an active glycerolated virus and 2 cc. of human immune serum. The incubation period was unusually long, *viz.* 30 days; the course of the disease was also unusually slow, since the animal did not become prostrate until the 38th day after injection. When the first count was made on the 37th day after injection both legs were flaccid, and the left arm was weak. On the following day, when the animal became prostrate, another count was made. Other counts were made on the 41st and 47th days after injection, which were the 11th and 17th days of the disease. The animal succumbed on the 49th day, and microscopic lesions of poliomyelitis were found in the brain and cord.

Monkeys 3 and 4 were inoculated intracerebrally with an incubated mixture of 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of an active glycerolated virus and 2 cc. of streptococcus immune rabbit serum 10 days before the blood

counts were made. Monkey 3 had been prostrate 2 days and Monkey 4 3 days at this time. Both animals were etherized on the 14th day, and autopsy showed characteristic lesions of poliomyelitis.

*Series III.*—Blood counts were made on twelve monkeys before inoculation with active virus, during the incubation period, and during the acute stage of poliomyelitis. Observations were made at intervals until the animals were killed by etherization or died of respiratory paralysis. All injections of the virus were intracerebral except in Monkeys 8 and 9, which received the virus by the nasal route on the day following an intraspinal injection of 2 cc. of normal horse serum. Nos. 5, 6, and 7 were from the Philippines, monkeys apparently closely similar to *Macacus cynomolgus*. The remainder were *Macacus rhesus*. The results are recorded in Table III. The variations in the circulating lymphocytes in Monkeys 5, 6, 7, and 8 are graphically represented in Text-fig. 1, *a*, *b*, *c*, and *d*, those of Monkeys 9 to 16 in Text-figs. 2 to 4.

*Series IV.*—Counts on five monkeys (Nos. 17 to 21), which received active virus by several routes but which did not develop the disease, are tabulated in Table IV. Fresh virus was given to Monkey 17 by mouth, to Monkey 18 by means of a nasal plug, to Monkey 19 by intrasciatic injection, and to Monkeys 20 and 21 by intracerebral inoculation.

*Series V.*—A summary of counts at intervals on monkeys during the acute stage and when partial or complete recovery had taken place is given in Table V. The fluctuations of the circulating lymphocytes are graphically represented in Text-figs. 5 and 6.

Monkey 22 of this series was injected intracerebrally with cultivated virus, first generation,[6] 4 days before the first blood count was made. At this time convulsions and ataxia were noted. On the 7th day after inoculation, when the third white blood count was made, the animal was partially paralyzed, but never became prostrate. 15 days after symptoms were first noted, at the time of the last blood count, the animal had almost completely recovered.

Monkey 23 was exposed to x-rays during the interval between the first and second blood counts, and the blood exhibited the characteristic lymphocytic drop.[7] Seven doses of unfiltered x-rays of 6 Holzknecht units each were given over

[6] Smillie, W. G., *J. Exp. Med.*, 1918, xxvii, 319.

[7] Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, xxix, 53.

TABLE III.

*Blood Counts on Monkeys during the Incubation Period after Injections of Poliomyelitic Virus.*

| Monkey No. | Day of observation. | Length of time after inoculation. | Day of symptoms. | Total lymphocytes. | | Total polymorphonuclear neutrophilic leucocytes. | |
|---|---|---|---|---|---|---|---|
| | | *days* | | *per cent* | | *per cent* | |
| 5* | 1 | | | 24.0 | 6,780 | 74.7 | 21,103 |
| | 3 | 2 | | 33.4 | 6,430 | 66.0 | 12,705 |
| | 4 | 3 | | 47.7 | 7,966 | 51.7 | 8,643 |
| | 6 | 5 | 1 | 12.7 | 2,937 | 86.7 | 20,049 |
| | 7 | 6 | 2 | 44.7 | 9,834 | 54.3 | 11,946 |
| | 8 | 7 | 3 | 38.0 | 6,983 | 61.0 | 11,204 |
| | 9 | 8 | 4 | 20.7 | 5,988 | 79.3 | 22,938 |
| | 11 | 10 | 6 | 15.3 | 4,777 | 85.3 | 26,635 |
| | 14 | 13 | 9 | 12.3 | 3,795 | 85.3 | 26,465 |
| 6* | 1 | | | 22.0 | 6,067 | 77.3 | 21,223 |
| | 3 | 2 | | 27.0 | 3,692 | 72.3 | 9,887 |
| | 4 | 3 | | 11.7 | 2,533 | 88.3 | 19,117 |
| 7* | 1 | 3 | | 34.7 | 10,315 | 61.3 | 18,221 |
| | 2 | 4 | | 41.0 | 4,797 | 47.7 | 5,581 |
| | 4 | 6 | 1 | 10.0 | 1,948 | 90.0 | 17,528 |
| 8† | 1 | | | 49.7 | 8,225 | 47.3 | 7,828 |
| | 2 | | | 51.0 | 6,464 | 45.3 | 5,742 |
| | 5 | | | 38.0 | 6,441 | 58.3 | 9,882 |
| | 9 | 4 | | 60.7 | 8,270 | 38.3 | 5,218 |
| | 11 | 6 | | 15.7 | 4,887 | 84.3 | 26,238 |
| | 13 | 8 | 1 | 42.3 | 7,254 | 56.0 | 9,604 |
| | 14 | 9 | 2 | 30.0 | 5,670 | 67.3 | 12,720 |
| 9† | 1 | | | 47.7 | 9,922 | 49.7 | 10,347 |
| | 12 | 2 | 11 | 32.0 | 5,040 | 66.0 | 9,735 |
| | 13 | 3 | 12 | 38.4 | 5,126 | 60.7 | 13,230 |
| | 14 | 4 | 13 | 27.4 | 4,226 | 72.0 | 11,106 |
| | 15 | 5 | 14 | 40.7 | 4,701 | 58.0 | 6,699 |
| | 16 | 6 | 15 | 30.0 | 3,833 | 68.7 | 8,776 |
| | 18 | 8 | 17 | 30.0 | 4,583 | 70.0 | 10,693 |
| | 19 | 9 | 18 | 16.0 | 2,524 | 83.7 | 13,204 |

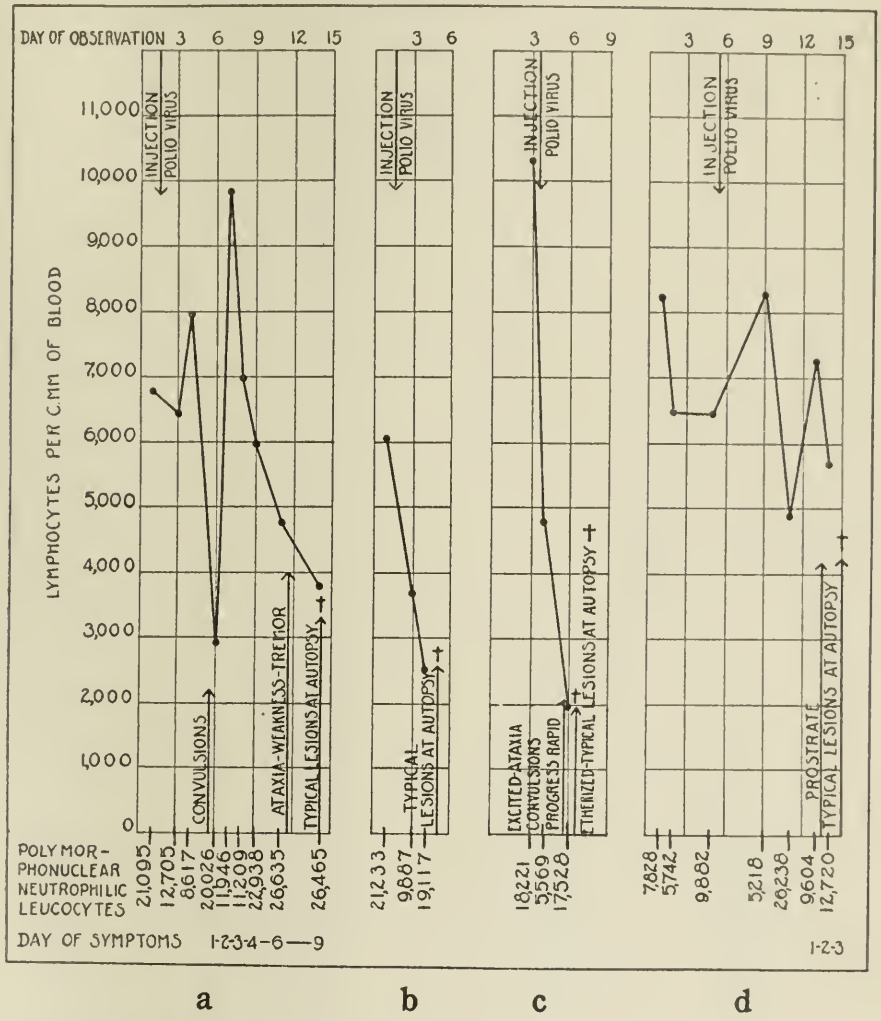
* Philippine monkey.

† Nasal route of infection. Previous intraspinal injection of 2 cc. of normal horse serum.

TABLE III—*Concluded.*

| Monkey No. | Day of observation. | Length of time after inoculation. | Day of symptoms. | Total lymphocytes. | | Total polymorphonuclear neutrophilic leucocytes. | |
|---|---|---|---|---|---|---|---|
| | | *days* | | *per cent* | | *per cent* | |
| 10 | 1 | 4 | | 32.0 | 10,696 | 54.7 | 18,214 |
| | 3 | 6 | | 51.5 | 15,939 | 47.1 | 14,578 |
| | 5 | 8 | 1 | 36.7 | 4,936 | 62.0 | 8,339 |
| | 6 | 9 | 2 | 23.0 | 3,835 | 73.7 | 12,290 |
| | 7 | 10 | 3 | 32.0 | 4,304 | 64.7 | 8,702 |
| | 8 | 11 | 4 | 29.0 | 3,567 | 68.7 | 8,450 |
| | 9 | 12 | 5 | 62.0 | 6,417 | 37.3 | 3,861 |
| | 10 | 13 | 6 | 33.7 | 4,457 | 66.3 | 8,768 |
| 11 | 1 | 2 | | 46.7 | 12,117 | 49.3 | 12,793 |
| | 2 | 3 | | 47.7 | 9,051 | 46.7 | 8,861 |
| | 5 | 6 | | 44.0 | 8,217 | 53.7 | 10,029 |
| | 6 | 7 | 1 | 13.0 | 3,452 | 86.7 | 23,019 |
| 12 | 1 | | | 57.7 | 15,348 | 34.0 | 9,044 |
| | 3 | 2 | | 60.0 | 10,740 | 37.0 | 6,623 |
| | 4 | 3 | | 78.0 | 11,661 | 19.0 | 2,841 |
| | 5 | 4 | | 69.3 | 19,477 | 28.3 | 3,870 |
| | 7 | 6 | | 64.4 | 13,347 | 35.3 | 7,316 |
| | 8 | 7 | 1 | 19.7 | 6,161 | 79.7 | 24,926 |
| 13 | 1 | | | 37.3 | 12,476 | 62.3 | 20,839 |
| | 3 | 2 | | 48.7 | 10,373 | 49.3 | 10,501 |
| | 6 | 5 | | 70.7 | 9,297 | 28.7 | 3,744 |
| | 7 | 6 | | 29.7 | 14,323 | 69.3 | 23,420 |
| | 8 | 7 | | 36.7 | 5,633 | 63.0 | 9,671 |
| | 9 | 8 | 1 | 18.7 | 3,675 | 81.0 | 15,917 |
| 14 | | | | 38.7 | 9,298 | 57.7 | 13,862 |
| | 8 | 7 | | 22.4 | 3,489 | 76.7 | 11,946 |
| | 11 | 10 | 2 | 10.0 | 2,373 | 89.3 | 21,186 |
| | 12 | 11 | 3 | 20.0 | 2,970 | 80.0 | 11,880 |
| | 13 | 12 | 4 | 14.3 | 2,431 | 82.3 | 13,991 |
| 15 | 1 | 3 | | 58.7 | 12,151 | 40.3 | 8,342 |
| | 4 | 6 | | 46.7 | 17,046 | 51.7 | 18,871 |
| | 7 | 9 | 2 | 24.7 | 3,557 | 72.3 | 10,411 |
| | 8 | 10 | 3 | 17.7 | 3,615 | 79.3 | 16,197 |
| 16 | 1 | 4 | | 45.0 | 8,179 | 53.3 | 9,687 |
| | 5 | 8 | 1 | 22.0 | 2,794 | 76.7 | 9,741 |
| | 6 | 9 | 2 | 14.7 | 2,132 | 83.7 | 12,137 |
| | 7 | 10 | 3 | 13.0 | 1,940 | 86.3 | 12,945 |
| | 8 | 11 | 4 | 11.0 | 2,540 | 87.0 | 20,097 |

a period of 6 days, the dorsal and ventral surfaces of the body being alternately exposed. Each dose was governed by the following factors: spark-gap 3 inches, milliamperes 10, distance from the target of the Coolidge tube to the skin 12 inches, and time of exposure 4 minutes. After these treatments the animal was inoculated intracerebrally with active virus and became completely prostrate



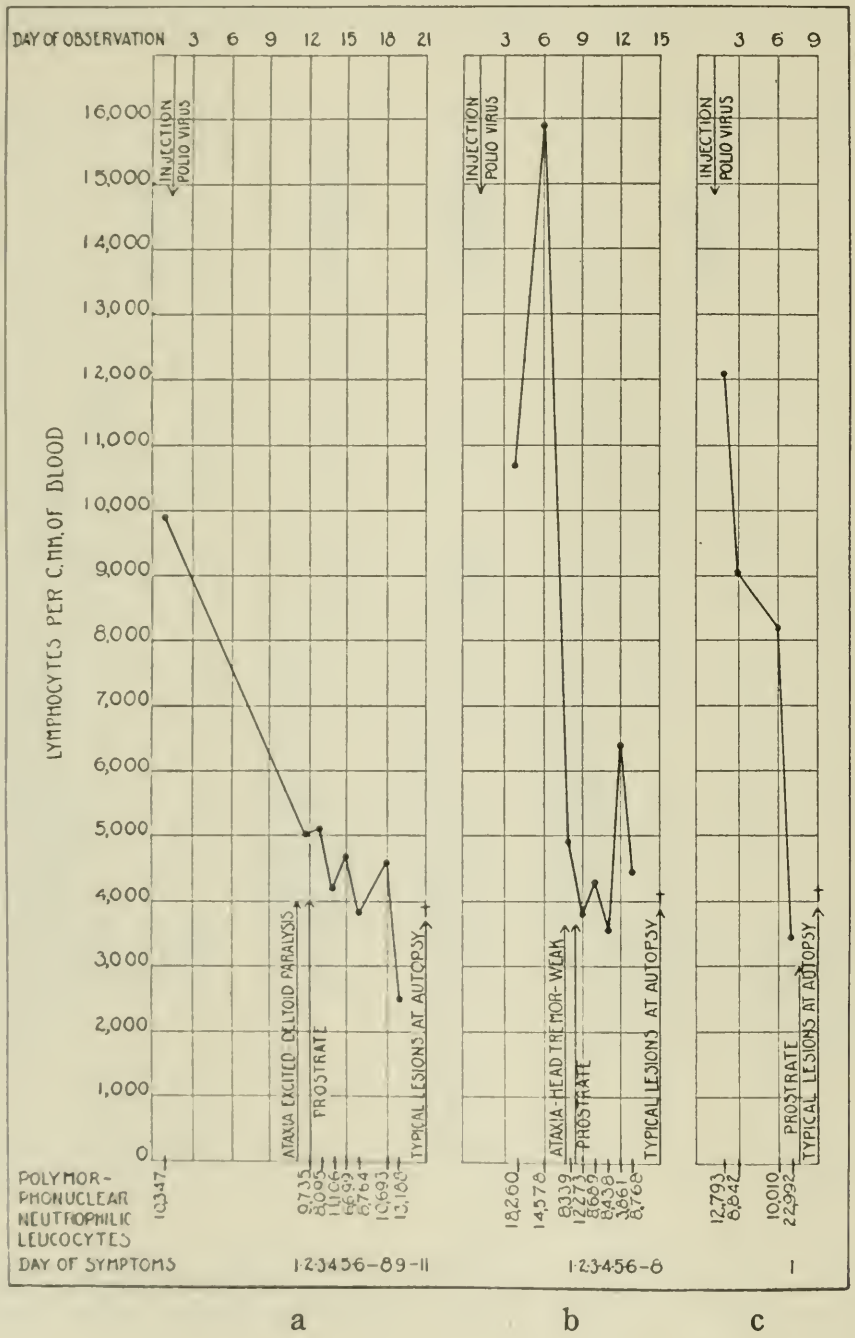

a b c d

TEXT-FIG. 1, *a*, *b*, *c*, and *d*. Lymphocyte curve of Monkeys 5, 6, 7, and 8 (Table III).

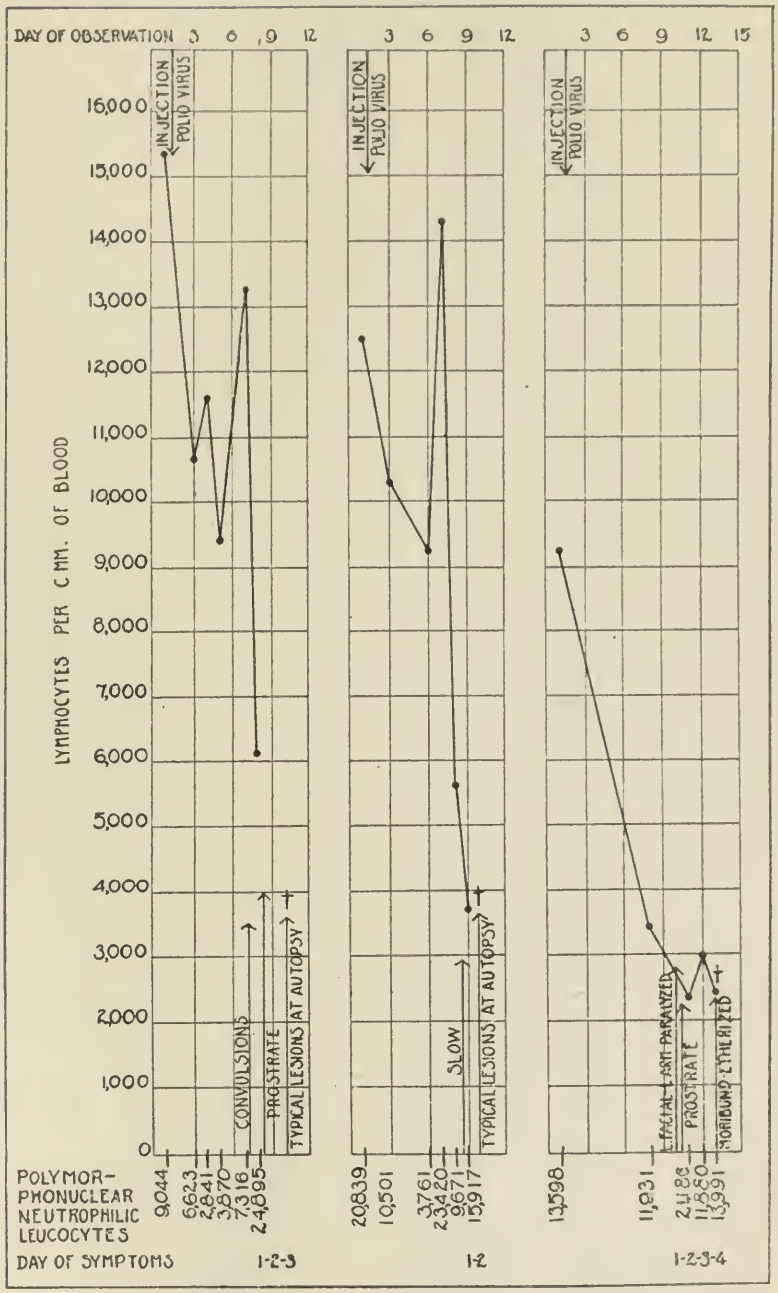
8 days later. The animal recovered with marked residual paralysis of both legs. Counts were made on the 59th day of observation, 45 days after prostration, and on the 76th day of observation.

Counts were made on Monkey 24 before injection and 5 days afterward. The animal was prostrate on the 12th day. Counts were made on the 16th, 28th, and 172nd days.

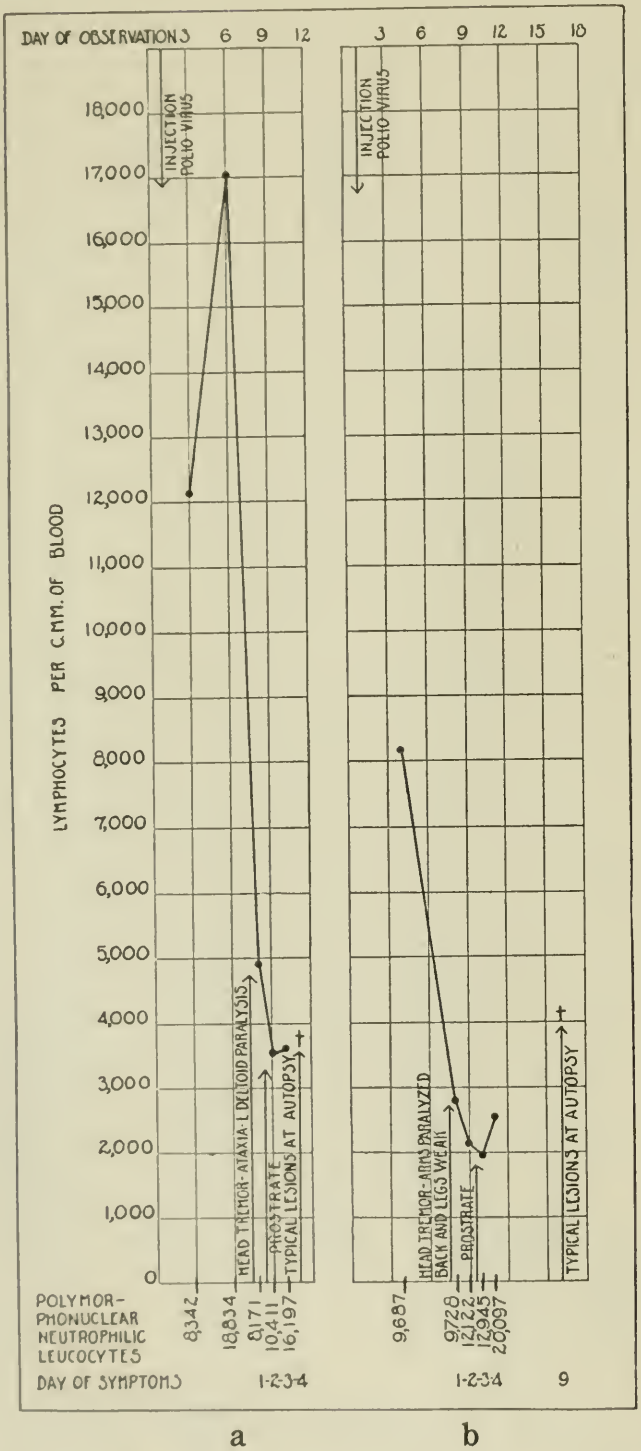
The first count was made on Monkey 25, 16 days after prostration, and the subsequent lymphocytic curve was followed for 22 days, during recovery with residual paralysis.




TEXT-FIG. 2, *a*, *b*, and *c*. (*a*) Lymphocyte curve of Monkey 9 (Table III). (*b*) Lymphocyte curve of Monkey 10 (Table III). (*c*) Lymphocyte curve of Monkey 11 (Table III).




TEXT-FIG. 3, *a*, *b*, and *c*. (*a*) Lymphocyte curve of Monkey 12 (Table III). (*b*) Lymphocyte curve of Monkey 13 (Table III). (*c*) Lymphocyte curve of Monkey 14 (Table III).




TEXT-FIG. 4, *a* and *b*. (*a*) Lymphocyte curve of Monkey 15 (Table III). (*b*) Lymphocyte curve of Monkey 16 (Table III).

TABLE IV.

*Blood Counts on Monkeys Which Received Virus but Did Not Develop Symptoms.*

| Monkey No. | Day of observation. | Length of time after inoculation. | Day of symptoms. | Total lymphocytes. | | Total polymorphonuclear neutrophilic leucocytes. | |
|---|---|---|---|---|---|---|---|
| | | *days* | | *per cent* | | *per cent* | |
| 17 | 1 | | | 41.0 | 9,615 | 57.0 | 12,367 |
| | 6 | 5 | | 51.0 | 8,096 | 46.3 | 7,360 |
| | 13 | 12 | | 31.4 | 4,561 | 64.7 | 9,398 |
| | 23 | 22 | | 54.4 | 8,772 | 35.0 | 5,644 |
| | 37 | 36 | | 46.7 | 8,556 | 45.0 | 8,556 |
| 18 | 1 | | | 50.7 | 11,205 | 40.3 | 8,906 |
| | 3 | 1 | | 56.4 | 9,292 | 39.7 | 6,541 |
| | 6 | 4 | | 49.3 | 8,849 | 47.0 | 8,437 |
| | 8 | 6 | | 55.0 | 16,871 | 42.3 | 12,976 |
| | 11 | 9 | | 68.3 | 16,871 | 28.3 | 6,055 |
| | 18 | 16 | | 56.7 | 17,393 | 39.7 | 12,178 |
| | 27 | 25 | | 55.4 | 11,426 | 43.0 | 8,869 |
| | 32 | 30 | | 59.4 | 15,622 | 38.7 | 10,178 |
| | 35 | 33 | | 47.7 | 9,922 | 49.7 | 10,347 |
| 19 | 1 | | | 43.3 | 9,959 | 51.7 | 11,891 |
| | 6 | 4 | | 54.0 | 10,139 | 42.0 | 7,886 |
| | 8 | 6 | | 42.7 | 7,163 | 54.7 | 9,178 |
| | 11 | 9 | | 56.7 | 9,242 | 39.0 | 6,357 |
| | 13 | 11 | | 50.0 | 7,188 | 43.3 | 6,224 |
| | 21 | 19 | | 54.7 | 6,687 | 43.0 | 5,257 |
| | 31 | 29 | | 43.0 | 7,740 | 56.3 | 10,134 |
| | 39 | 37 | | 54.7 | 8,971 | 43.0 | 7,052 |
| 20 | 1 | | | 66.0 | 16,320 | 23.7 | 5,824 |
| | 3 | 2 | | 71.3 | 11,889 | 26.3 | 4,386 |
| | 4 | 3 | | 72.0 | 13,392 | 21.7 | 4,036 |
| | 5 | 4 | | 66.0 | 9,719 | 27.7 | 4,079 |
| | 7 | 6 | | 64.0 | 14,848 | 30.3 | 7,030 |
| | 8 | 7 | | 62.0 | 10,804 | 31.7 | 5,524 |
| | 9 | 8 | | 56.7 | 9,667 | 34.7 | 5,916 |
| | 11 | 10 | | 58.0 | 8,715 | 36.7 | 5,514 |
| | 15 | 14 | | 74.3 | 16,718 | 20.3 | 4,568 |
| | 18 | 17 | | 59.3 | 10,674 | 36.0 | 6,480 |
| | 22 | 21 | | 66.7 | 14,724 | 25.0 | 5,519 |
| | 26 | 25 | | 74.3 | 13,764 | 18.0 | 3,334 |
| | 33 | 32 | | 79.7 | 15,362 | 16.3 | 3,142 |
| | 36 | 35 | | 72.3 | 16,792 | 23.3 | 5,178 |

TABLE IV—*Concluded.*

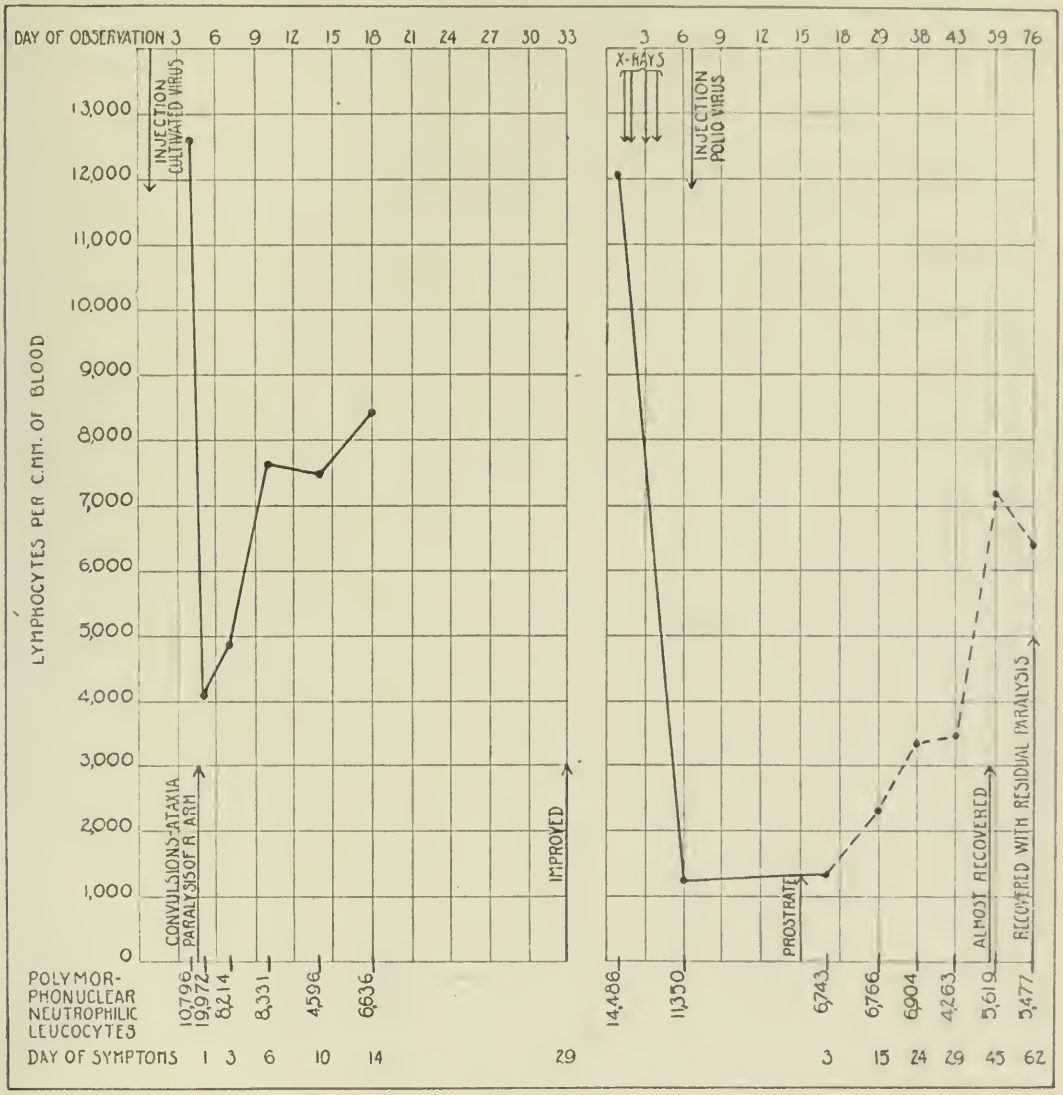
| Monkey No. | Day of observation. | Length of time after inoculation. | Day of symptoms. | Total lymphocytes. | | Total polymorphonuclear neutrophilic leucocytes. | |
|---|---|---|---|---|---|---|---|
| | | *days* | | *per cent* | | *per cent* | |
| 21 | 1 | | | 61.0 | 11,087 | 33.0 | 5,998 |
| | 2 | 1 | | 30.7 | 4,313 | 66.3 | 9,315 |
| | 7 | 6 | | 45.3 | 6,670 | 47.7 | 6,024 |
| | 8 | 7 | | 62.3 | 7,507 | 35.0 | 4,218 |
| | 11 | 10 | | 60.3 | 9,105 | 36.7 | 5,542 |
| | 14 | 13 | | 67.3 | 11,592 | 30.7 | 5,288 |
| | 17 | 16 | | 76.7 | 10,086 | 22.7 | 2,985 |
| | 21 | 20 | | 64.0 | 10,304 | 34.0 | 5,474 |

TABLE V.

*Blood Counts on Monkeys Observed during the Process of Recovery from Poliomyelitis.*

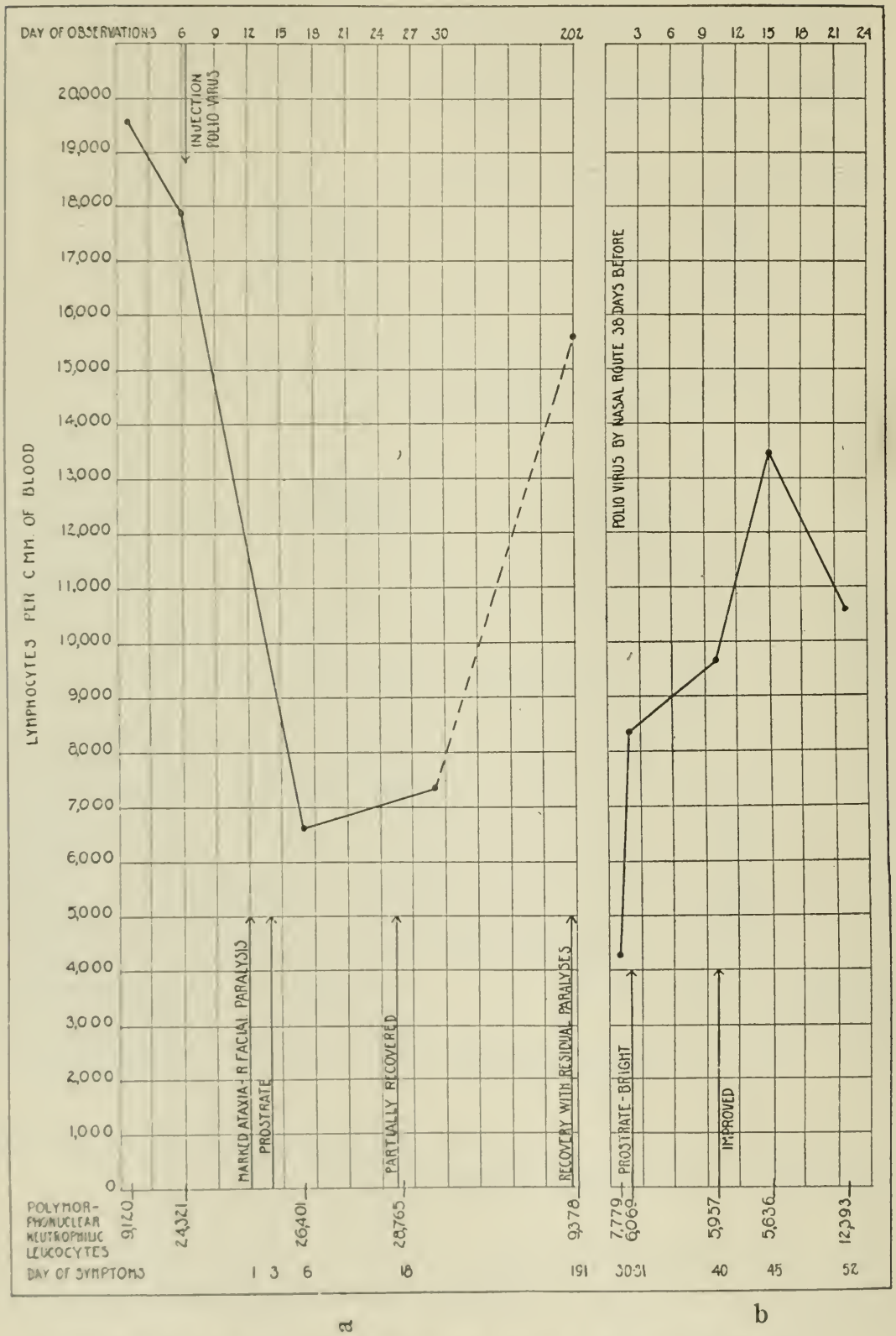
| Monkey No. | Day of observation. | Length of time after inoculation. | Day of symptoms. | Total lymphocytes. | | Total polymorphonuclear neutrophilic leucocytes. | | Remarks. |
|---|---|---|---|---|---|---|---|---|
| | | *days* | | *per cent* | | *per cent* | | |
| 22 | 1 | 4 | 1 | 55.3 | 12,619 | 45.7 | 10,820 | Convulsions; ataxia. |
| | 2 | 5 | 2 | 16.7 | 4,087 | 81.7 | 11,996 | |
| | 4 | 7 | 4 | 36.3 | 4,864 | 61.3 | 8,214 | Paralyzed but not prostrate. |
| | 7 | 10 | 7 | 29.3 | 7,640 | 70.3 | 18,331 | |
| | 11 | 14 | 11 | 62.3 | 7,476 | 38.3 | 4,596 | |
| | 15 | 18 | 15 | 55.0 | 8,429 | 43.3 | 6,636 | Almost recovered. |
| 23 | 1 | | | 45.0 | 12,071 | 54.0 | 14,486 | X-rayed after count. |
| | 6 | | | 9.0 | 1,231 | 83.0 | 11,350 | |
| | 17 | 11 | 3 | 16.0 | 1,332 | 81.0 | 6,743 | Prostrate 3 days. |
| | 29 | 23 | 15 | 23.3 | 2,318 | 68.0 | 6,766 | Partly recovered. |
| | 38 | 32 | 24 | 29.3 | 3,355 | 60.3 | 6,904 | |
| | 43 | 37 | 29 | 42.3 | 3,485 | 50.3 | 4,263 | |
| | 59 | 53 | 45 | 53.7 | 7,236 | 41.7 | 5,619 | Almost recovered. |
| | 76 | 70 | 62 | 50.3 | 6,363 | 43.3 | 5,477 | Recovered with residual paralysis. |
| 24 | 1 | | | 65.0 | 19,565 | 30.3 | 10,033 | |
| | 6 | 5 | | 41.7 | 17,889 | 56.7 | 24,324 | |
| | 17 | 16 | 4 | 20.0 | 6,625 | 79.7 | 26,401 | Prostrate 3 days. |
| | 29 | 28 | 16 | 20.0 | 7,340 | 76.3 | 29,528 | |
| | 173 | 172 | 160 | 57.7 | 15,593 | 34.7 | 9,378 | Recovered with residual paralysis. |
| 25 | 1 | 30 | 16 | 29.3 | 3,289 | 69.3 | 7,779 | Prostrate. |
| | 2 | 31 | 17 | 53.7 | 7,357 | 44.3 | 6,069 | |
| | 10 | 39 | 25 | 56.7 | 8,661 | 39.0 | 5,957 | |
| | 15 | 44 | 30 | 67.0 | 12,462 | 30.3 | 5,636 | |
| | 22 | 51 | 37 | 41.7 | 9,570 | 54.0 | 12,393 | Partial recovery with residual paralysis. |

*Series VI.*—In Table VI are given blood counts made on monkeys which had completely recovered from acute poliomyelitis and proved immune to subsequent inoculations of active virus. All the animals




TEXT-FIG. 5, *a* and *b*. (*a*) Lymphocyte curve of Monkey 22 (Table V). (*b*) Lymphocyte curve of Monkey 23 (Table V).

had some residual contractures. Recovery, in the animals of this group, was of long duration, varying from 1 to 3 months at the time of the first blood count.




TEXT-FIG. 6, *a* and *b*. (*a*) Lymphocyte curve of Monkey 24 (Table V). (*b*) Lymphocyte curve of Monkey 25 (Table V).

TABLE VI.

*Blood Counts on Monkeys Which Had Recovered from Acute Symptoms before the First Count.*

| Monkey No. | Day of observation. | Length of time since recovery. | Total lymphocytes. | | Total polymorphonuclear neutrophilic leucocytes. | |
|---|---|---|---|---|---|---|
| | | *days* | *per cent* | | *per cent* | |
| 26 | 1 | 72 | 52.7 | 8,550 | 43.7 | 7,090 |
| | 2 | 73 | 36.3 | 6,561 | 61.3 | 11,080 |
| | 5 | 76 | 55.0 | 6,463 | 40.3 | 4,735 |
| 27 | 1 | 72 | 57.7 | 7,746 | 28.0 | 3,759 |
| | 2 | 73 | 66.7 | 7,270 | 26.3 | 2,967 |
| | 5 | 76 | 72.0 | 7,200 | 20.0 | 2,000 |
| 28 | 1 | 92 | 50.7 | 4,700 | 45.7 | 4,277 |
| | 2 | 93 | 38.0 | 17,733 | 59.7 | 12,149 |
| | 5 | 96 | 43.3 | 6,712 | 54.0 | 8,370 |
| 29 | 1 | 84 | 46.3 | 7,281 | 47.3 | 7,438 |
| | 2 | 85 | 43.3 | 5,932 | 53.7 | 7,357 |
| | 6 | 89 | 40.3 | 8,181 | 52.3 | 10,617 |
| | 8 | 91 | 44.0 | 8,965 | 51.3 | 10,452 |
| | 9 | 92 | 52.7 | 7,879 | 42.7 | 6,384 |
| | 16 | 99 | 33.7 | 7,515 | 61.3 | 13,670 |
| | 20 | 103 | 42.3 | 8,238 | 50.3 | 9,796 |
| | 35 | 118 | 43.3 | 8,335 | 52.3 | 10,068 |
| 30 | 1 | 31 | 73.0 | 16,608 | 21.7 | 4,937 |
| | 2 | 32 | 67.0 | 10,318 | 29.0 | 4,466 |
| | 5 | 35 | 71.7 | 16,168 | 16.7 | 3,766 |
| | 16 | 46 | 39.0 | 14,182 | 60.3 | 20,381 |
| | 20 | 50 | 79.3 | 24,524 | 16.3 | 5,040 |

## DISCUSSION.

All the blood counts made on monkeys during the course of typical acute experimental poliomyelitis show a variation from the normal. This change is apparent in Table VII,[8] in which the average lymphocytes and polymorphonuclear counts and percentage during the acute stage are compared with normal averages. There are included aver-

[8] Counts on Monkey 6 (Table III) are not included in the computation.

ages at the time of the highest and also of the lowest lymphocyte count. The variations are sufficiently great to warrant definite conclusions.

After injection of active poliomyelitic virus the lymphocytes are diminished but return to their former number and are actually increased between the 4th and 6th days of the incubation period (Table III). The polymorphonuclear count is high at this time. The normal average of lymphocytes of 11,815 (Table I) is increased to an average of 19,696 though the average percentage is slightly lowered. During the first 3 days after onset a marked diminution in the lymphocytes takes place. Thus, instead of an average normal lymphocytic count of 11,815, the number is 3,302, and the average percentage

TABLE VII.

*Average Variation in White Cells during Infection with Poliomyelitis.*

| Status of monkeys at time of counts. | No. of animals studied. | Total No. of counts. | Large and small lymphocytes. Average. | Average total No. of lymphocytes per c.mm. | Average total No. of polymorphonuclear neutrophilic leucocytes. | |
|---|---|---|---|---|---|---|
| | | | *per cent* | | *per cent* | |
| Normal | 40 | 121 | 53.7 | 11,815 | 41.1 | 9,116 |
| Incubation period at time of highest lymphocytic count | 12 | 12 | 44.0 | 19,696 | 54.3 | 13,383 |
| In acute stage at time of lowest lymphocytic count | 12 | 12 | 15.6 | 3,302 | 83.7 | 18,231 |
| Prostrate | 4 | 6 | 21.9 | 2,758 | 76.5 | 10,800 |
| Recovered with residual paralysis | 6 | 22 | 53.2 | 9,026 | 41.3 | 6,800 |

is 15.6. At this time the polymorphonuclear neutrophilic leucocytes are materially increased in number (18,231) as compared with an average normal of 9,116, and the percentage is also increased from an average of 41 to 84 per cent of the total white cells When the monkeys become completely prostrate metabolism is at low ebb, and there occurs a further decrease in the actual number of lymphocytes (Table III) which remains low for long periods (Tables II and V). The total number of polymorphonuclear neutrophilic leucocytes returns to normal, but there remains a relative increase averaging 77 per cent. Finally, during recovery both types of cells return to the average normal count and relation. No stimulation of the lympho-

cytes above normal appears during recovery. The counts made on one animal (Monkey 24, Table V) at 160 days after onset are almost the same as those made before the injection of virus.

In the monkeys receiving virus but not developing symptoms there seemed to be a constant tendency for the lymphocytes to decrease following the administration of the virus (Table IV). This decrease was followed by a gradual return to normal.

Monkey 23 (Table V) was x-rayed before inoculation with the virus of poliomyelitis, and the return of the circulating lymphocytes to their normal level, in this animal, seemed to be considerably delayed. As the x-rays have a depleting effect on the circulating lymphocytes[9] similar to that exhibited by the virus of poliomyelitis, the protracted recovery may, in this instance, depend on a summative action of the two agents.

Monkey 22 (Table V) was also included in the series used by Smillie[6] in attempts to produce poliomyelitis with cultivated virus. Typical pathological lesions were not observed at autopsy; however, the symptoms and the blood curve simulating those observed in known poliomyelitic monkeys are suggestive and offer possible additional proof that the monkey developed mild poliomyelitis after intracerebral inoculation with the fourth generation of a culture of the globoid bodies and recovered before it was etherized for autopsy.

## SUMMARY AND CONCLUSIONS.

Blood counts were made on six series of monkeys before and at various intervals after the injection of active poliomyelitic virus. From the data thus obtained the variations in the circulating white cells have been followed in the several stages of the disease (*a*) before injection, (*b*) during the incubation period, (*c*) during the acute stage, including the stage of prostration, and (*d*) during recovery. On account of experimental conditions not all the monkeys were observed during the four periods, so that averages of counts in several monkeys are used for comparison.

Averages of 121 counts on 40 normal monkeys are recorded.

[9] Amoss, H. L., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 115.

Immediately following the injection of the virus the relative and actual numbers of lymphocytes are slightly diminished. In many cases the curve continues sharply downward. In others from the 4th to the 6th day there is an actual increase for a brief period to a point somewhat in excess of the original count. With the onset of symptoms a lymphocytic crisis takes place. The curve then continues slightly downward, while the polymorphonuclear neutrophilic leucocytes are relatively and actually increased at approximately the same time. During the stage of prostration the curve of the polymorphonuclear neutrophilic leucocytes returns to almost normal, while the lymphocytic curve continues slightly downward. With recovery the lymphocytes slowly return to normal after several weeks. There is no evidence of lymphocytic stimulation after recovery.

Eosinophilic, basophilic, large mononuclear, and transitional leucocytes follow the depressions and stimulations exhibited by the neutrophilic cells of the same series.

The results here recorded are consistent with the observations of Peabody, Draper, and Dochez on human cases.

The increase in the total number of circulating lymphocytes after the lymphocytic crisis is coincident with the passing of the acute stage.

Additional evidence is presented to indicate that Smillie produced atypical but definite poliomyelitis in the monkey with cultivated virus.

# EFFECTS OF LARGE DOSES OF X-RAYS ON THE SUSCEPTIBILITY OF THE MONKEY TO EXPERIMENTAL POLIOMYELITIS.

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The ready communicability of poliomyelitis among human beings is generally accepted save by a certain few who disregard the idea of selective susceptibility. It is a matter of common observation that of many persons of the most susceptible age definitely exposed only a small percentage contracts the disease. For example, in the New York City epidemic of 1916 there were approximately 1,200,000 individuals of the more susceptible ages, *i.e.* less than 16 years,[1] exposed, but only 8,750 of this age in a total of 9,023 cases were reported. In the epidemic in Westchester County single cases in families of several children were the rule, although in uncommon instances there were three or four cases in the same family. This contrasts strongly with the experience in measles, a disease of practically no selective action. The reasons for the differential power of poliomyelitis to attack are not apparent; however, three facts have been lately adduced which may have some bearing on the problem. In the first place, it has been shown[2] that the route of infection with the virus by the blood stream in monkeys, which is ordinarily closed, except when massive doses are used, can be traversed by smaller doses after intraspinal injection of substances setting up an aseptic meningitis. Infection by the nasal route is also rendered much easier by similar preparation. Flexner and Amoss[3] suggest that possibly one reason for infection or non-

[1] The epidemic of poliomyelitis (infantile paralysis) in New York City in 1916, Department of Health of the City of New York, Monograph series, No. 16, New York, 1917.

[2] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

[3] Flexner and Amoss, *J. Exp. Med.*, 1917, xxv, 525.

infection in persons exposed may lie in the patency or continuity, as the case may be, of the meningeal-choroidal complex. Secondly, Zingher[4] has shown that whereas only 30 per cent of apparently normal children yield a positive reaction to intradermal injection of diphtheria toxin, about 80 per cent of persons recently recovered from poliomyelitis give a positive reaction. This suggests either a general state of lowered resistance, as indicated by susceptibility to both poliomyelitis and diphtheria, or less probably that infection with the former reduces resistance to the latter. Thirdly, Amoss and Taylor[5] noted the power of nasal washings from certain individuals to neutralize the virus of poliomyelitis and suggest that this action may be the first line of defense against poliomyelitic infection.

No definite data have been presented to indicate variations in resistance to poliomyelitic virus among monkeys, except perhaps the impression that tuberculous animals seem to be more susceptible. Moreover, the conditions of the experimental infection are purposely arranged to reduce the chance of variations in individual resistance. The virus when it has once been adapted to the monkey becomes highly infective and remains so during many animal transfers. Finally, however, after several years passage the virulence diminishes.[6] The original M. A. strain adapted to monkeys by Flexner and Lewis[7] in 1909 now possesses less infective power and lends itself to observations on susceptibility in experimental poliomyelitis.

The observations of Peabody, Draper, and Dochez[8] on human cases and of Taylor[9] in experimental poliomyelitis with regard to the remarkable lymphocytic changes accompanying the infection and the focal infiltrations of these cells as one of the histological characteristics of the disease suggest an intimate relation between the infection and the circulating lymphocytes. It may be possible by bring-

[4] Zingher, A., *Dept. Health, City of New York, Reprint Series, No. 52*, 1917.

[5] Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, xxv, 507.

[6] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 195.

[7] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 1639.

[8] Peabody, F. W., Draper, G., and Dochez, A. R., A clinical study of acute poliomyelitis, Monograph of The Rockefeller Institute for Medical Research, No. 4, New York, 1912.

[9] Taylor, H. D., *J. Exp. Med.*, 1919, xxix, 97.

ing about conditions in which the lymphocytes are greatly diminished to render the monkey more susceptible to poliomyelitic virus. The experiments here recorded support this belief.

## EXPERIMENTAL.

By exposure to properly controlled doses of x-rays it is possible to diminish the circulating lymphocytes.[10] Accordingly, two normal monkeys were selected for each experiment. One was treated with x-rays[11] daily or every other day until the total number of lymphocytes per c.mm. of blood was between 1,000 and 2,000. A Berkefeld filtrate of a 5 per cent suspension of glycerolated M. A. virus was injected intracerebrally into the x-rayed and the control animal. The dose of virus was previously titrated and known to be subinfective. Should the dose of the virus be too large, both animals are infected, and if the maximum subinfective dose is reduced by more than one-half, the more susceptible x-rayed animal does not respond with an attack of poliomyelitis.

Three series of experiments were carried out according to the plan outlined. The protocols follow.

*Series I.—Macacus rhesus* A (control). Jan. 17, 1917. Blood count, 7,750 lymphocytes and 3,950 polymorphonuclear neutrophilic leucocytes per c.mm. Injected intracerebrally with 1 cc. of a Berkefeld filtrate of a 5 per cent suspension of glycerolated poliomyelitic monkey cord (M. A. virus). The monkey remained well.

*Macacus rhesus* B. Jan. 3, 1917. Blood count, 6,930 lymphocytes and 8,465 polymorphonuclear neutrophilic leucocytes per c.mm. Received seven doses of x-rays, 6 Holzknecht units each. Jan. 15. Blood count, 1,257 lymphocytes and 4,300 polymorphonuclear neutrophilic leucocytes per c.mm. (Text-fig. 1, *a*). Jan. 17. Received intracerebrally (at the same time as the control injection) 1 cc. of the Berkefeld filtrate of M. A. virus used in Monkey A. Jan. 23. Excitable; left arm paralyzed; right deltoid weak. Jan. 24. Prostrate. Jan. 27. Moribund; etherized.

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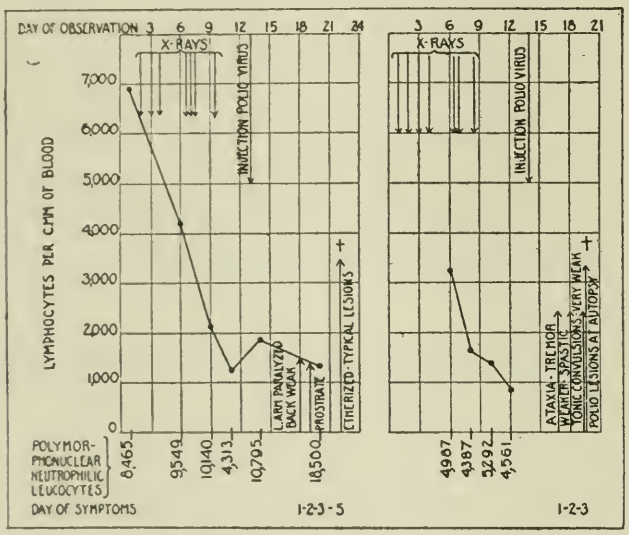
[10] Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, xxix, 53.

[11] Unfiltered x-rays of 6 Holzknecht units each were given at each dose alternately on the dorsal and ventral surfaces. Each dose was governed by the following factors: spark-gap 3 inches, milliamperes 10, distance from the target of the Coolidge tube to the skin 12 inches, and time of exposure 4 minutes.

*Autopsy.*—Severe acute lesions of poliomyelitis, perivascular infiltration, and necrosis of ganglion cells.

*Series II.—Macacus rhesus* C (control). Feb. 5, 1917. Blood count showed 16,630 lymphocytes and 4,050 polymorphonuclear neutrophilic leucocytes per c.mm. Injected intracerebrally with 1 cc. of a Berkefeld filtrate of a 5 per cent suspension of glycerolated poliomyelitic monkey cord (M. A. virus). The monkey remained well. Mar. 5. Began daily treatments with x-rays. After seven doses the lymphocytes were reduced in number to 878 per c.mm., but no symptoms of poliomyelitis developed. 6 months later the animal showed symptoms of tuberculosis and died.

*Autopsy.*—Marked tuberculosis of lungs and abdominal viscera.




a b

TEXT-FIG. 1, *a* and *b*. (*a*) Lymphocytic curve of Monkey B, Series I. (*b*) Lymphocytic curve of Monkey D, Series II.

*Macacus rhesus* D. Blood was not counted before treatment with x-rays. On Feb. 5, 1917, seven doses of x-rays had been given, and the blood count was as follows: lymphocytes 885 (15 per cent), polymorphonuclear neutrophilic leucocytes 4,561 (77.3 per cent) (Text-fig. 1, *b*). On this date there was injected intracerebrally 0.75 cc. of the Berkefeld filtrate of M. A. virus given Monkey C (control). Feb. 8. Ataxic; protects right leg; marked head tremor. Feb. 9. Weaker; ataxia increased. Feb. 10, a.m. Back and both legs weak; convulsion lasting 2 minutes. 2 p.m. Found dead.

*Autopsy.*—All the organs very pale; spleen very small. Brain and cord showed intense congestion of gray matter. Microscopic examination showed focal infiltrations and perivascular infiltration of cells, most of which were polymorphonuclear leucocytes. The lesions in the cord and spinal ganglia were not marked.

*Macacus rhesus* E. Blood count before exposure to x-rays, 8,785 (43.6 per cent) lymphocytes and 8,180 (40.6 per cent) polymorphonuclear neutrophilic leucocytes. Received eight doses of x-rays. Feb. 5, 1917. The blood count was as follows: 581 (9 per cent) lymphocytes and 4,810 (74.6 per cent) polymorphonuclear leucocytes. Injected intracerebrally with 0.5 cc. of the poliomyelitic virus filtrate. The animal never showed symptoms of poliomyelitis.

In this experiment the control showed no symptoms after receiving 1 cc. of the virus filtrate, while the animal treated with x-rays, receiving only 0.75 cc., succumbed to poliomyelitis. The control animal after 28 days was treated with x-rays, but no symptoms developed, indicating the destruction or removal of the virus in that time.

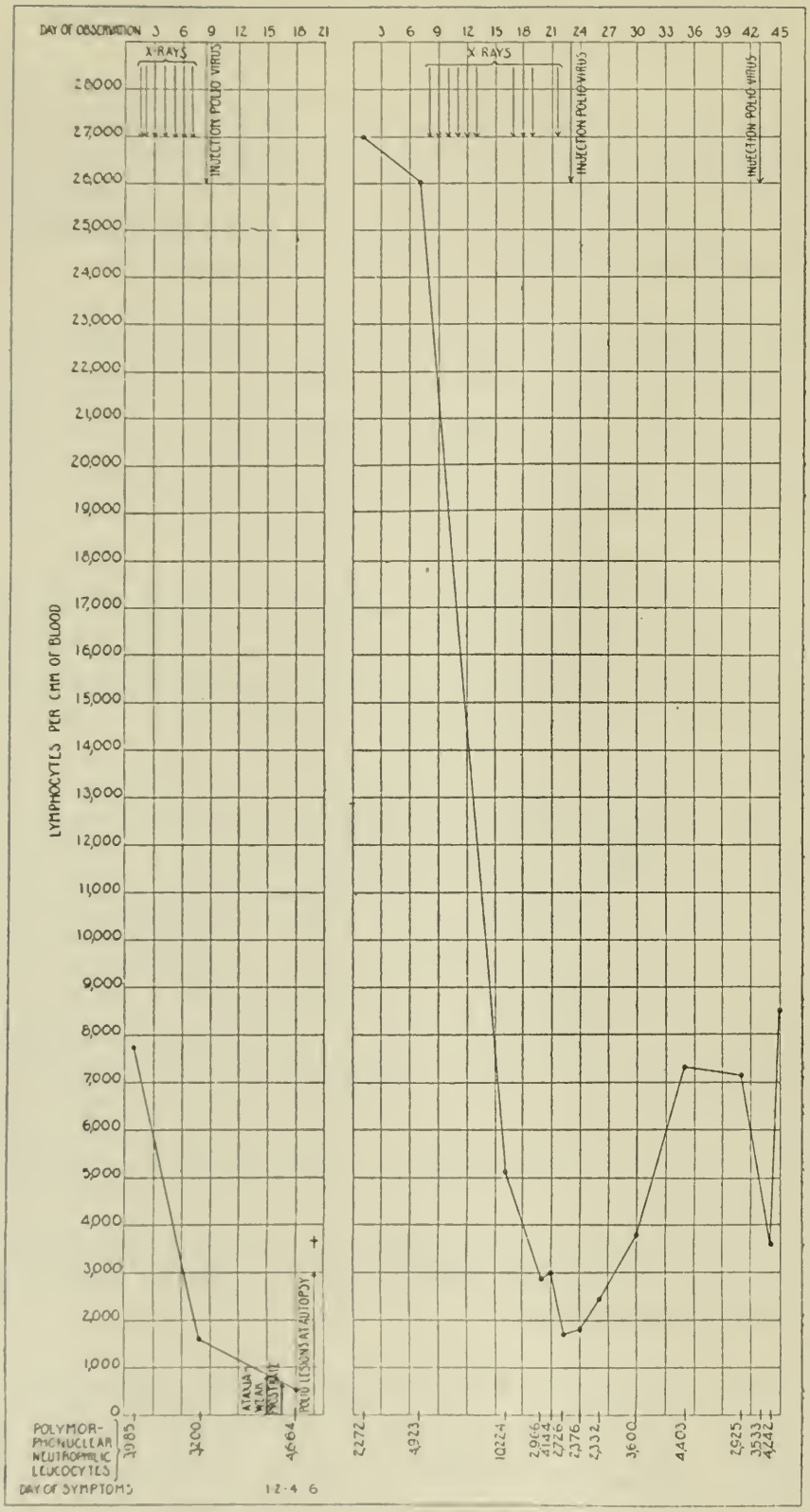
*Series III.—Macacus rhesus* F (control). Apr. 3, 1918. Blood count, lymphocytes 8,432 (42 per cent) and polymorphonuclear neutrophilic leucocytes 11,162 (55.6 per cent) per c.mm. Injected intracerebrally with 0.5 cc. of a Berkefeld filtrate of a 5 per cent suspension of glycerolated poliomyelitic monkey cord (M. A. virus). The animal showed no symptoms of poliomyelitis, but later died of tuberculosis.

*Autopsy.*—Almost total involvement of left lung and tuberculosis of abdominal viscera. There was no evidence of poliomyelitic lesion in the brain or cord.

*Macacus rhesus* G. Mar. 26, 1918. Blood count, 7,751 (59.7 per cent) lymphocytes and 3,985 (30.3 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. Received daily treatments with x-rays, 6 Holzknecht units each, for 7 days. Apr. 3. Blood count, 1,625 (14.7 per cent) lymphocytes and 3,200 (75.3 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. (Text-fig. 2, *a*). Injected intracerebrally with 0.5 cc. of the Berkefeld suspension of the glycerolated poliomyelitic cord (M. A. virus) given Monkey F. Apr. 10. Ataxic; head tremor; partial paralysis of left arm, right deltoid, and back. Apr. 11. Completely prostrate. Apr. 13. Blood count, 520 (10 per cent) lymphocytes and 4,664 (89.7 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. Apr. 15. Found dead.

*Autopsy.*—Slight perivascular infiltration and nerve cell degeneration in the medulla and slight focal accumulations of cells, most of which were polymorphonuclear neutrophilic leucocytes.

The outcome of this experiment supports the findings in Series I, in which the control animal remained unaffected by a dose of M. A. poliomyelitic virus which caused complete paralysis in a monkey which had been treated with x-rays with a consequent decrease in the circulating lymphocytes.




TEXT-FIG. 2, *a* and *b*. (*a*) Lymphocytic curve of Monkey G, Series III. (*b*) Lymphocytic curve of Monkey I.

*Survival of a Subinfective Dose of the Virus in Normal Monkey Brain.*

The usual incubation period in experimental poliomyelitis is from 7 to 10 days; however, when a weak virus[6] is used, or in neutralization experiments[5] this period may be prolonged to 30 or 40 days. An incubation period longer than 30 days is unusual. It is presumed that the virus lay dormant for the prolonged period or multiplied slowly. Multiple injections of virus weakened by long storage in 50 per cent glycerol[12] apparently lessen rather than increase the resistance of the monkey to subsequent injections, if the interval between injections is about 7 days. Longer periods have not been studied. In monkey poliomyelitic brain removed from the body and placed under anaerobic conditions at 37°C. in tissue ascitic fluid the virus may survive without multiplication for 20, but not for 30 days.[13] The virus does not survive 7 days *in vivo* in rabbit brain[14] or even for 2 days in the rat brain.[15] Is the subinfective dose then disposed of in a monkey of normal resistance within the maximum incubation period? The following experiments have a bearing on this question.

*Experiment 1.*—Monkey C, used as control in Series II, received 1 cc. of virus filtrate intracerebrally. When 28 days had elapsed the animal was exposed to x-rays until the lymphocytes had decreased to 878 per c.mm. The animal had no symptoms of poliomyelitis.

*Experiment 2.—Macacus rhesus* H. Mar. 23, 1917. Received 1 cc. of filtrate of suspension of virus used in Experiment 1 and at the end of 15 days was exposed to x-rays, which reduced the lymphocytes to 1,125 per c.mm. No symptoms of poliomyelitis developed in the animal.

Apparently in the animals of normal resistance subinfective doses are disposed of in 15 days or are so weakened that reduction in resistance of the animal does not permit of infection. The experiments were not carried further because of lack of monkeys due to war conditions.

[12] Flexner and Amoss, *J. Exp. Med.*, 1917, xxv, 539.
[13] Flexner and Amoss, *J. Exp. Med.*, 1915, xxi, 509.
[14] Amoss, H. L., *J. Exp. Med.*, 1918, xxvii, 443.
[15] Amoss, H. I ., and Haselbauer, P., *J. Exp. Med.*, 1918, xxviii, 429.

*Is Immunity Once Established Reduced by Exposure to X-Rays?*

Flexner and Lewis[16] have shown that monkeys which have passed through an attack of poliomyelitis are immune to subsequent injections of an active virus. Moreover, sera from immune monkeys and from human beings[17] who have had the disease possess definite neutralizing power for the poliomyelitic virus. It is probable that the immunity could not be destroyed by any of the factors accompanying exposure to x-rays. A single experiment was carried out on a monkey paralyzed by previous injections of poliomyelitic virus.

*Experiment 3.—Macacus rhesus* I (active immune) had received dried poliomyelitic virus on Feb. 10, 1917, showed symptoms on Feb. 21, and had complete paralysis of both legs and deltoid muscles on Feb. 23. The animal gradually improved with residual paralysis of both legs. Apr. 6. Blood count, 26,062 (81 per cent) lymphocytes and 4,920 (15.3 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. X-ray treatments were begun. During the succeeding 14 days ten exposures, of 6 Holzknecht units each, were made. Apr. 24. The blood count was as follows: 1,739 (37 per cent) lymphocytes and 2,726 (58 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. Apr. 25. Injected intracerebrally with 1 cc. of a 5 per cent suspension of fresh poliomyelitic monkey cord. No symptoms developed. May 15. Injected intracerebrally with 2 cc. of 5 per cent suspension of active poliomyelitic monkey cord. The animal remained as before without any increase in paralysis or other symptoms of poliomyelitis. The lymphocytic changes are graphically recorded in Text-fig. 2, *b*.

In this experiment exposure to x-rays sufficient to reduce the circulating lymphocytes to one-fifteenth of their original number failed to destroy immunity in the monkey established by a previous attack of poliomyelitis.

## SUMMARY AND CONCLUSIONS.

In two series of the experiments here recorded the monkey which had been repeatedly exposed to x-rays responded with typical acute poliomyelitis to an intracerebral inoculation of poliomyelitic virus fil-

[16] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, lv, 662.

[17] Levaditi and Landsteiner, *Compt. rend. Soc. biol.*, 1910, lxviii, 311. Römer, P. H., and Joseph, K., *Münch. med. Woch.*, 1910, lvii, 568. Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780. Anderson, J. F., and Frost, W. H., *J. Am. Med. Assn.*, 1911, lvi, 663.

trate, whereas the normal control receiving the same dose showed no symptoms. In another series the x-rayed animal came down with typical poliomyelitis after inoculation with three-fourths of the dose which was not infective for the control. It has been demonstrated that the x-rays diminished both the number of circulating lymphocytes and the resistance of the animal to the weak poliomyelitic virus. Whether the lowered resistance of the animals as the result of the treatment with x-rays is due to the reduction of circulating lymphocytes in each of the x-rayed monkeys is not determined in these experiments.

However, the great reduction in lymphocytes in human cases[8] and in monkeys during the acute stage of experimental poliomyelitis[9] and the gradual return of the cells to their former numbers during recovery strongly suggest a definite relation between these cells and one factor of resistance in poliomyelitis.

On the other hand, the reduction in resistance by x-rays, while definite, is not sufficiently great to warrant the conclusion that we are dealing with major factors governing infection or non-infection.

Another experiment in this paper deals with the survival of a subinfective dose of the virus in the normal monkey brain. A monkey receiving the subinfective dose of the virus was exposed to x-rays at 28 days, another at 15 days after injection, but neither animal showed symptoms of poliomyelitis. It is concluded that within this period the virus did not remain unchanged in the normal monkey brain.

An attempt to reduce the immunity in a monkey acquired by an attack of experimental poliomyelitis was unsuccessful.

# THE ACTION OF CHLORINATED ANTISEPTICS ON BLOOD CLOT.

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Experiments recently reported from this laboratory[1] demonstrated that hypochlorite solutions, in the concentration and alkalinity used clinically, exhibit a solvent action on necrotic tissue. Fiessinger and his coworkers[2] have reported a similar action of these solutions on pus cells. The importance of the latter observation is emphasized by the experiments of Rous and Jones,[3] which demonstrated that intact leucocytes may protect bacteria which they have phagocyted from the action of an antiseptic. These bacteria, when liberated after autolysis of the leucocytes, are viable and may proliferate in an apparently normal manner.

As the hypochlorite solutions are extensively used in the treatment of infected wounds, and as their cleansing properties have been emphasized, it seemed important to determine definitely whether these solutions were effective in dissolving or penetrating blood clot. The possibility that clotted blood in wounds might serve as a protective covering for virulent microorganisms, thereby preventing the bactericidal action of the antiseptic employed, seemed worthy of investigation.

As it is generally recognized, and as early experiments seemed to demonstrate conclusively, that solutions of a degree of concentration and alkalinity compatible with clinical use exhibited little solvent action on blood clot, certain modifications were later introduced in an endeavor to influence the solutions in this direction.

[1] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155. Austin, J. H., and Taylor, H. D., *ibid.*, 627.

[2] Fiessinger, N., Moiroud, P., Guillaumin, C.-O., and Vienne, G., *Ann. méd.*, 1916, iii, 133.

[3] Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 601.

*Method.*—Rabbit blood was allowed to clot in test-tubes, and after separation from the serum the remaining cylindrical mass was cut into a number of discs, each 1 cm. in thickness and 1.5 cm. in diameter. Each disc was then placed in 50 cc. of the solution to be tested for varying lengths of time. The appearance and size of the discs were noted before and after exposure to the action of the solution and examined carefully for any evidence of dissolution or penetration by the antiseptics. Careful controls were included in every experiment.

## EXPERIMENTAL.

*Experiment 1.*—The first series of experiments was of a morphological nature, and an effort was made to determine by the appearance of the blood clot discs whether or not any solvent action was demonstrable. Dakin's hypochlorite (0.5 per cent sodium hypochlorite), chloramine-T (2 per cent), and dichloramine-T (5 per cent in chlorcosane) solutions apparently had no solvent, disintegrative, or penetrative action on the discs when allowed to act for periods of time varying from 15 minutes to 12 hours.

*Experiment 2.*—In the hope of varying the permeability of the blood clots, they were first treated with $\frac{M}{8}$ calcium chloride and $\frac{M}{8}$ sodium chloride. Loeb[4] has already shown that calcium chloride increases and sodium chloride decreases the permeability of masses of finely powdered gelatin. After varying lengths of time in the salt solutions (from 15 minutes to 2 hours), the clots were transferred to the antiseptic solutions, hypochlorite 0.5 per cent, chloramine-T 2 per cent, dichloramine-T 5 per cent in chlorcosane, and in distilled water, and allowed to remain in contact with these solutions for from 15 minutes to 12 hours. No appreciable action was noticeable except all absence of hardening of the surface layer of the clot first treated with calcium chloride and later transferred to Dakin's hypochlorite solution. It was then decided to test the penetration of the solutions in a manner allowing objective analysis, and the following experiments were performed in the hope of getting accurate and comparable figures with regard to the relative solvent powers of certain antiseptics.

*Experiment 3.*—10 cc. of sterile rabbit blood were placed in a sterile test-tube containing 0.5 cc. of a 24 hour bouillon culture of *Staphylococcus aureus* and shaken thoroughly against a sterile rubber stopper to insure even distribution of the bacteria through the resulting clot. After clotting and separation of the serum the cylindrical mass was cut, with precautions against further contamination, into equal sized discs, 1 cm. in thickness and 1.5 cm. in diameter. The discs were then placed in bottles containing 50 cc. of the following solutions: (1) Dakin's hypochlorite solution 0.5 per cent; (2) chloramine-T solution 2 per cent; and (3)

[4] Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343.

sterile salt solution, as control. A disc was removed from each bottle after a half hour and a second after a 1 hour interval. The discs were then washed in two changes of sterile saline solution to remove all traces of the test solution; the second saline wash contained a few drops of a $\frac{N}{10}$ sodium thiosulfate solution to neutralize any chlorine remaining. Each disc was thoroughly ground in a sterile mortar with 5 cc. of saline solution, agar tubes were inoculated with two loopfuls of the resulting emulsion, and plates poured. After 24 hours incubation at 37°C. the colonies developing in the plates were counted, and the results are summarized in Table I.

TABLE I.

| Time of contact. | Dakin's solution. | Chloramine-T solution. | Salt solution control. |
|---|---|---|---|
| *hrs.* | | | |
| $\frac{1}{2}$ | 60 | 100 | Confluent. |
| 1 | 60 | 100 | " |

*Experiments 4 and 5.*—These experiments were similar to Experiment 3, and the results are recorded in Tables II and III.

TABLE II.

| Time of contact. | Dakin's solution. | Chloramine-T solution. | Salt solution control. |
|---|---|---|---|
| *hrs.* | | | |
| $\frac{1}{2}$ | 300 | 312 | Confluent. |
| 1 | 280 | Confluent. | " |
| 2 | Confluent. | 300 | " |

TABLE III.

| Time of contact. | Dakin's solution. | Chloramine-T solution. | Salt solution control. |
|---|---|---|---|
| *hrs.* | | | |
| 1 | Confluent. | Confluent. | Confluent. |
| 2 | " | " | " |

*Experiment 6.*—Discs prepared as in Experiments 3 to 5 were placed in $\frac{M}{8}$ calcium chloride and $\frac{M}{8}$ sodium chloride for from 1 to 2 hours and later transferred to Dakin's solution to test the effect of the saline solution on the permeability of the clot. The results are given in Table IV.

TABLE IV.

| Solution 1. | Time of contact. | Solution 2. | Time of contact. | No. of colonies. |
|---|---|---|---|---|
| | *hrs.* | | *hrs.* | |
| M/8 calcium chloride. | 1 | Dakin's hypochlorite. | 2 | 222 |
| M/8 " " | 1 | | 0 | Confluent. |
| Dakin's hypochlorite. | 2 | | 0 | 800 |
| M/8 sodium chloride. | 1 | Dakin's hypochlorite. | 2 | 545 |
| M/8 " " | 1 | | 0 | Confluent. |

*Experiment 7.*—The same experiment was repeated, but in one series (A) the clot discs nearest the top of the clotted blood were used and in the other (B) the clot discs nearest the bottom were used. This was done in order to rule out, as far as possible, inaccuracies due to the uneven distribution of the bacteria in the cylindrical clot. The results of both series are given in Table V.

TABLE V.

| Solution 1. | Time of contact. | Solution 2. | Time of contact. | No. of colonies. | |
|---|---|---|---|---|---|
| | | | | A | B |
| | *hrs.* | | *hrs.* | | |
| Dakin's hypochlorite. | 2 | | | 15 | 20 |
| M/8 calcium chloride. | 1 | Dakin's hypochlorite. | 2 | 20 | 40 |
| M/8 sodium " | 1 | " " | 2 | 10 | 40 |
| 9 per cent sodium chloride (normal). | 3 | | | 10 | |

*Experiment 8.*—To insure still further even distribution of the bacteria through the blood clot the following variation was instituted. A rabbit was injected in-

TABLE VI.

| Solution 1. | Time of contact. | Solution 2. | Time of contact. | No. of colonies. |
|---|---|---|---|---|
| | *hrs.* | | *hrs.* | |
| M/8 sodium chloride. | 2 | | | Confluent. |
| M/8 calcium " | 2 | | | " |
| Dakin's 0.5 per cent hypochlorite. | 2 | | | " |
| M/8 sodium chloride. | 1 | Dakin's 0.5 per cent hypochlorite. | 2 | " |
| M/8 calcium " | 1 | Dakin's 0.5 per cent hypochlorite. | 2 | " |

travenously with 15 cc. of a 24 hour bouillon culture of *Staphylococcus aureus* and 1 minute later bled from the heart. The blood was allowed to clot in the usual manner and discs were prepared as described above. The discs were placed in solutions, taken out, ground as described above, inoculated into agar tubes, and later poured in plates, as in previous experiments. The results are shown in Table VI.

*Experiments 9 and 10.*—The same experiment was repeated on two later occasions with the results shown in Tables VII and VIII.

TABLE VII.

| Solution 1. | Time of contact. | Solution 2. | Time of contact. | No. of colonies. |
|---|---|---|---|---|
| | *hrs.* | | *hrs.* | |
| M/8 sodium chloride. | 2 | | | 50 |
| M/8 calcium " | 2 | | | 200 |
| Dakin's 0.5 per cent hypochlorite. | 2 | | | 50 |
| M/8 sodium chloride. | 1 | Dakin's 0.5 per cent hypochlorite. | 2 | 100 |
| M/8 calcium " | 1 | Dakin's 0.5 per cent hypochlorite. | 2 | 50 |

TABLE VIII.

| Solution 1. | Time of contact. | Solution 2. | Time of contact. | No. of colonies. |
|---|---|---|---|---|
| | *hrs.* | | *hrs.* | |
| M/8 sodium chloride. | 2 | | | Confluent. |
| Chloramine-T 2 per cent. | 2 | | | " |
| Dakin's 0.5 per cent hypochlorite. | 2 | | | " |
| Eusol (0.5 per cent hypochlorite). | 2 | | | " |
| M/8 sodium chloride. | 1 | Chloramine-T 2 per cent. | 2 | " |
| M/8 " " | 1 | Dakin's 0.5 per cent hypochlorite. | 2 | " |
| M/8 " " | 1 | Eusol (0.5 per cent hypochlorite). | 2 | " |

*Experiment 11.*—Equal sized discs of blood clot, through which *Staphylococcus aureus* had been equally distributed by the *intra vitam*, intravenous injection of the organisms in the usual manner, were placed first in a trypsin solution and later in Dakin's solution to determine whether the trypsin, by partial disruption of the

clot, rendered it more permeable to the antiseptic solution. A 1 per cent solution of commercial trypsin and Dakin's solution with a hypochlorite concentration of 0.5 per cent were used. The results of this experiment are given in Table IX.

TABLE IX.

| Solution 1. | Time of contact. | Solution 2. | Time of contact. | No. of colonies. |
|---|---|---|---|---|
| | *hrs.* | | *hrs.* | |
| Trypsin 1 per cent. | 2 | Dakin's solution. | 2 | Confluent. |
| " 1 " " | 24 | " " | 2 | " |
| Saline solution. | 2 | | | " |

Inasmuch as blood clot had been found so resistant to hypochlorite solutions which readily dissolve necrotic tissue, pus cells, and other organic matter, a series of experiments was performed to determine, if possible, the resistant constituent of the clot. The results are summarized in the description of Experiment 12.

*Experiment 12.*—(*a*) Plasma clot was prepared by centrifuging at high speed freshly drawn rabbit blood. The resulting supernatant, clear fluid quickly coagulated. This clot, without corpuscles, is readily dissolved by Dakin's hypochlorite solution having a sodium hypochlorite concentration of 0.5 per cent. 2 per cent chloramine-T solution is without such action.

(*b*) Red blood corpuscles, prepared by defibrinating freshly drawn rabbit blood and adding sufficient saline solution to make a 5 per cent suspension of cells, are readily dissolved by the hypochlorite solution. The chloramine-T solution merely laked the corpuscles without exhibiting solvent action.

(*c*) Pus cells, prepared from human exudate and from dogs in response to the irritant action of aleuronat, are dissolved by the hypochlorite but not by the chloramine-T solution.

(*d*) It seemed probable that the fibrin of blood clot was more resistant than the fibrin of the plasma clot because the fibrin is held together in closer mechanical mass by the blood cells than in the more loosely formed plasma.[5]

[5] Experiments were undertaken but proved inconclusive and were interrupted by Dr. Taylor's death.

## SUMMARY.

This work demonstrates that the chlorinated antiseptics have no power to penetrate blood clots and destroy bacteria therein contained. Correspondingly, blood clots may protect virulent bacteria for a long period of time and the organisms properly planted will be able to proliferate in a normal manner.[6,7]

[6] It seems probable that the fibrin of the blood clot is the resistant substance as plasma and red and white cells are easily dissolved by these antiseptics.

[7] The work on this paper up to this point had been written up by Dr. Taylor before his death. No attempt has been made to discuss his clear-cut and conclusive experiments. The summary given here is taken from the quarterly report rendered to the Director of The Rockefeller Institute.

# THE TROPISTIC ACTION OF BLOOD VESSELS ON THE MIGRATION OF CHROMATOPHORES.

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(Received for publication, October 29, 1918.)

Loeb[1] has shown that the chromatophores appearing in the yolk sac early in the development of *Fundulus heteroclitus* are attracted to the blood vessels when there is an active circulation. Thus the little red and black cells, each with many ramifying processes, begin to migrate from their scattered positions in the yolk sac to the blood vessels of this organ only after the active heart is propelling the blood through the vessels. If potassium chloride, in quantities sufficient to stop the heart beat and circulation, is added to the water containing the developing embryos, the chromatophores develop normally, but until the 4th day, at least, there is no migration comparable with that observed in controls where by this time the pigment cells are definitely approaching the vessels. Loeb suggests that there is probably something present in the circulating blood which is responsible for this tropistic phenomenon.

The experiments recorded below were devised for the purpose of arriving at a satisfactory explanation of the phenomenon, if possible.[2]

*Experiment 1.*—60 eggs of *Fundulus heteroclitus*, artificially fertilized, were kept in sea water. After 4 days incubation black and red chromatophores, with widely spread out processes were scattered over the yolk sac. By this time the hearts had begun to beat and the blood was circulating through the thin walled blood vessels which formed a network over the surface of the yolk sac. None of the chromatophores showed any relation to the vessels at this time. On the 6th day of incubation, however, the chromatophores had approached and embraced the blood vessels of the yolk sac so that the location of the vessels could be accurately determined by the coloring of their periphery caused by the closely

[1] Loeb, J., *J. Morphol.*, 1893, viii, 161; On the heredity of the marking in fish embryos, Biological lectures, Boston, 1899, 227.

[2] These experiments were suggested by Dr. Jacques Loeb.

approximated black and not quite so closely approximated red chromatophores. Twenty of the eggs were allowed to remain in the sea water, twenty transferred to $\frac{M}{8}$ potassium chloride in sea water, and twenty to $\frac{M}{16}$ potassium chloride in sea water. The results are summarized in Table I.

TABLE I.

| Length of time after fertilization. | Sea water. | | M/16 KCl | | Transferred from M/16 KCl to sea water.* | | Transferred from M/8 KCl to sea water.* | |
|---|---|---|---|---|---|---|---|---|
| | No. with active circulation. (Chromatophores on vessels.) | No. with inactive circulation. (Chromatophores spread.) | No. with active circulation. (Chromatophores on vessels.) | No. with inactive circulation. (Chromatophores spread.) | No. with active circulation. (Chromatophores on vessels.) | No. with inactive circulation. (Chromatophores spread.) | No. with active circulation. (Chromatophores on vessels.) | No with inactive circulation. (Chromatophores spread.) |
| *days* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 6 | 100 | 0 | 100 | 0 | | | | |
| 8 | 100 | 0 | 100 | 0 | | | | |
| 9 | 100 | 0 | 45 | 55 | | | | |
| 10 | 100 | 0 | 50 | 50 | 60 | 40 | 0 | 100 |
| 11 | 100 | 0 | 10 | 90 | 40 | 60 | 0 | 100 |
| 16 | All hatched swimming. | | 0 | 100 | 55 | 45 | 10 | 90 |
| 17 | | | 0 | 100 | 30 | 70 | 10 | 90 |
| 18 | | | 0 | 100 | 45 | 55 | 10 | 90 |
| 19 | | | 0 | 100 | 33 | 67 | 20 | 80 |
| 20 | | | 0 | 100 | 33 | 67 | 10 | 90 |
| 25 | | | All dead. | | 55 | 45 | 0 | 100 |
| 26 | | | | | 45 | 55 | 0 | 100 |
| 27 | | | | | 70 | 30 | 0 | 100 |
| 41 | | | | | 100 | 0 | All dead. | |
| 46 | | | | | 0 | 100 | | |

* Left in $\frac{M}{8}$ potassium chloride and $\frac{M}{16}$ potassium chloride for 4 days (from 6th to 10th day of incubation period) and then placed in sea water.

The chromatophores of all the embryos developing in sea water alone remained in close contact with the blood vessels until the time of hatching (16th day after fertilization). All hatched, and the fish were apparently normal.

In those left in $\frac{M}{16}$ potassium chloride the hearts and circulations gradually became inactive and as this occurred the chromatophores again became spread out on the surface of the yolk sac. None of these embryos ever hatched. Ten of the latter after 4 days in the $\frac{M}{16}$ potassium chloride (from the 6th to the 10th day of incubation)

were again returned to sea water without excess potassium. At that time 60 per cent still had active hearts and circulations, and the chromatophores were closely embracing the vessels. In 40 per cent the hearts had stopped, the blood was not circulating, and the chromatophores were spread out over the surface of the yolk sac. At the observations made after this time there were at various times different percentages of embryos with active hearts and circulations but always those in which the blood was flowing through the vessels also had the chromatophores in close contact with the blood vessels. Contrasting with this the chromatophores were always spread out over the surface of the yolk sac when the circulation was inactive. On the 41st day of incubation all had active circulation and chromatophores closely approximating the blood vessels. Thus on the 46th day of incubation, which was as far as the observations were continued, 100 per cent showed spread out chromatophores, and in none of these was there an active circulation.

In those left in $\frac{M}{8}$ potassium chloride and later transferred to sea water without excess potassium chloride a few again developed active circulations and in these the chromatophores again approximated and embraced the vessels in the characteristic manner. In those with inactive circulation, however, the chromatophores remained scattered and showed no relation to the blood vessels. When the circulation again stopped, in those that had begun a second time, the chromatophores again became spread out in the same manner.

A few confirmatory observations of interest may be briefly considered here.

1. When the circulation had only been inactive for 24 hours or less as in the column headed "Transferred from $\frac{M}{16}$ KCl to sea water," on the 11th day of incubation, it could be seen that the embryos in which the circulation had last stopped showed a much less pronounced wandering and spreading over of the chromatophores than those in which it had not started again.

2. The result was similar when the circulation had started but 24 hours or less before observation, in the column headed "Transferred from $\frac{M}{16}$ KCl to sea water," on the 18th day of incubation. Thus two of the three embryos in this category showed only partial approximation of the chromatophores to the vessels and not the complete stage noted in the remainder.

3. Sometimes there was an embryo present whose heart gave an occasional spasmodic beat, in the column headed "$\frac{M}{16}$ KCl," on the 11th day of incubation, and in these the chromatophores had not migrated far from the blood vessels and became now partially spread out. This is what would be expected if, as seems probable, this was the last in which the circulation had stopped.

4. In the embryos with beating heart but inactive circulation, in the column headed "Transferred from $\frac{M}{8}$ KCl to sea water," on the 16th day of incubation, there is usually a clump of chromatophores closely approximating the base of the beating heart where it is fixed to the surface of the yolk sac. It seems suggestive that chromatophores collect about the beating heart after they have left the vessels through which the blood has ceased to flow.

5. In other embryos with slow circulation following the removal of the stopped heart from $\frac{M}{8}$ potassium chloride to sea water, we find all stages in the restoration of the circulation and where this is rapid the chromatophores closely approximate the vessels, where slow they form but a loose meshwork about the vessels.

*Experiment 2.*—This experiment was in every way comparable with Experiment 1.

*Experiment 3.*—Eggs of the same teleost were allowed to develop normally until the 6th day when they were placed in flasks containing sea water to which had been added a few drops of a dilute solution of sodium cyanide (0.1 per cent). Eggs placed in 50 cc. of sea water to which two drops of the sodium cyanide solution had been added, when removed, at the end of 9 days, still had active circulations. The chromatophores were in about the same state as at the time when the eggs were first placed in the cyanide solution; *i.e.*, most were approximated and had embraced the vessels, but a few, especially those of the red variety, were still partially spread out over the surface of the yolk sac. At this time the controls, which had been kept in sea water without cyanide, had hatched. Those placed in the cyanide never hatched. When kept for 9 days in 50 cc. of sea water plus two drops of sodium cyanide the circulations were inactive but the hearts gave occasional spasmodic beats or beat steadily. The chromatophores had not spread out as they did when the circulation was stopped before the heart in Experiments 1 and 2. After removal from the cyanide to sea water or potassium chloride solutions the chromatophores behave exactly as they did under similar conditions in Experiments 1 and 2. The cyanide by lowering the rate of oxidation may have interfered with the motility of the chromatophores.

Experiments with alcohol were next tried.

Eggs fertilized 4 days previously were placed in sea water to which absolute ethyl alcohol had been added in sufficient quantity to make solutions of 2, 3, 4, and 5 per cent respectively. Thirty eggs were placed in each solution. After 18 hours ten eggs were removed from each solution and placed in sea water. The same procedure was repeated after 24 and again after 30 hours and each lot of eggs, separately kept, was observed at frequent intervals. Table II gives the results.

TABLE II.

| Day of incubation. | 2 per cent. | | | 3 per cent. | | | 4 per cent. | | | 5 per cent. | | | Control. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 18 hrs. | 24 hrs. | 30 hrs. | 18 hrs. | 24 hrs. | 30 hrs. | 18 hrs. | 24 hrs. | 30 hrs. | 18 hrs. | 24 hrs. | 30 hrs. | |
| 5 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | + | 0 | 0 | 0 | ++ |
| 6 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | + | + | 0 | 0 | ++ |
| 10 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ | + | 0 | ++ |
| 20 | H. | H. | H. | H. | H. | H. | H. | H. | H. | H. | + | D. | H. |
| 30 | | | | | | | | | | | + | | |

++, hearts and circulations active.
+, hearts but not circulations active.
0, neither hearts nor circulations active.
H., hatched.
D., dead.

In Table II majorities are indicated as the results were too irregular to record *in toto* without too much complexity. However, the results in each instance are similar to those encountered in previous experiments in every particular. Where the circulation is active (++), the chromatophores closely approximate the blood vessels and tightly embrace them. The embracing process is less complete the shorter the time since the circulation has again commenced; *i.e.*, 5 per cent, 10th day of incubation. When the heart is active but the circulation is at a standstill (+), the chromatophores are widely spread out over the yolk sac; *i.e.*, 5 per cent, 6th day of incubation. This is also the case when neither the heart nor the circulation is active (0); *i.e.*, 5 per cent, 5th day of incubation. There was considerable mortality among the embryos treated with alcohol, and in certain instances the circulations in some of the embryos were inactive while others were active. The tendency is indicated by the graphic record of the majority. When variations occurred there was always the consistent relation between the circulation and the chromatophores.

## DISCUSSION.

The distribution and arrangement of the chromatophores of the yolk sac of embryonic *Fundulus heteroclitus* seem to be determined by their relation to the most abundant supply of oxygen or to the lower hydrogen ion concentration. Thus, before there is any circulation they are spread out widely on the surface of the yolk sac without regard to the blood vessels. As soon as the circulation starts, the chromatophores approach the capillary, creeping longitudinally along the surface of, coupling up with those adjacent, and finally, forming an almost continuous covering of the vessels of the yolk sac. It may be presumed that at this time the tension of oxygen is higher and the hydrogen ion concentration lower in the circulating blood than in the tissue cells. If the circulation is stopped by any means, potassium chloride, sodium cyanide, ethyl alcohol, even with a beating heart, the chromatophores tend to spread out once more over the surface of the yolk sac in such a way that the greatest possible surface area is approximated to the sea water surrounding the embryo. With a beating heart, however, a clump of chromatophores is often found about the base of this organ, for reasons not entirely clear. In the experiments recorded above all stages in the development of a circulation and the cessation of one once begun have been encountered and it may be safely stated that there seems to be a direct relation between an active circulation and a close approximation and envelopment of the vessels by the chromatophores. A substance which, like sodium cyanide, inhibits oxidation also inhibits further migrations of the chromatophores, probably by paralyzing the latter; the tropistic reactions of the chromatophores are resumed when the poison (sodium cyanide) is removed and the eggs are transferred to a proper medium (sea water). When the eggs die the chromatophores shrivel up and appear as small dark spots against the opaque background of the egg. The red chromatophores respond much more slowly to an adequate stimulus than the black ones.

## CONCLUSIONS.

1. The migrations of the chromatophores of the yolk sac of *Fundulus heteroclitus* seem to be chemotropic in character.

2. The determining factor seems to be either a high tension of oxygen or a comparatively low concentration of hydrogen ions.

# BLOOD VOLUME IN WOUNDED SOLDIERS.

## I. Blood Volume and Related Blood Changes after Hemorrhage.

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### INTRODUCTION.

Our knowledge on the subject of hemorrhage and the subsequent restoration of the circulation is in a state of rapid change. New facts are constantly coming to light which necessitate revision of previous conceptions. Furthermore, the data at hand, although much increased of late, are by no means complete. This work is therefore presented with the realization that the interpretation of some of our findings may be open to question, and that as regards their significance further observations may lead to somewhat different conclusions. However, certain phenomena have occurred so consistently that it has seemed safe to make fairly definite deductions in these instances.

There is a lack of general agreement among investigators on problems of blood loss as to the data which are of most value in determining the actual condition of the patient after hemorrhage, and the ensuing changes which occur in his blood. The principal reason for this diversity of opinion appears to lie in the fact that all the methods heretofore employed in the estimation of blood changes have failed to take into account variations occurring in the volume of the blood. It is well recognized that following hemorrhage the body promptly begins to add fluid to the circulation in an attempt to make up the total blood bulk to its previous amount, but little actual knowledge

exists concerning the rapidity with which this diluting process occurs and the factors controlling it. Thus it is not surprising that the data given by red cell counts, hemoglobin per cent, hematocrit determinations, etc., when taken alone, are often difficult to interpret, since they indicate only the variations occurring in one unit of blood. These same data, however, when viewed side by side with blood volume determinations, take on an entirely different significance. Changes in hemoglobin and red cells, whose cause has heretofore seemed most obscure, become, in the light of this more complete knowledge, readily explainable and are seen to occupy their proper place in relation to a changing blood volume. For example, a marked drop in hemoglobin per cent following the rise produced by transfusion, has been generally regarded as an unfortunate occurrence due to hemolysis or perhaps to some less rapid destruction of the introduced cells. Repeated blood volume estimations have revealed the true nature of this phenomenon, showing that in such cases the volume was under normal, even after the transfusion, and that the drop in hemoglobin was due to progressive dilution of the blood as the volume was made up.

The present study includes a series of twenty-one cases of hemorrhage, which were observed as completely as circumstances would permit. Blood volume tests were made in fourteen cases, and with one or two exceptions the tests were performed repeatedly. The patients were of two types,—those suffering (*a*) from the late effects of primary hemorrhage and (*b*) from secondary blood loss. Infection was present in the majority of instances. The charts of those cases are shown which serve to illustrate best the various findings. Reference only is made to the other cases.

### *A. Initial Changes in Circulation after Hemorrhage.*

#### *1. Blood Volume.*

Blood volume determinations, following hemorrhage, made by the vital red method,[1] on a number of cases showed a surprising reduction

[1] This test was devised by Keith, Rowntree, and Geraghty. It has given very satisfactory results in our hands (Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, xvi, 547).

in the total blood bulk. Patients giving a history of loss of blood when first wounded, with a second hemorrhage several days to a week or more afterwards, as a rule had a volume of 60 per cent or less of the normal. Cases that reached the Base without a positive history of a severe bleeding had not infrequently a diminution in blood volume of 20 to 30 per cent. Text-figs. 1 and 2 (from Cases 1 and 2) are those of patients with secondary hemorrhage.[2] In Case 1 the initial determination, made within an hour after hemorrhage, showed a volume of 3,060 cc.—59 per cent of normal. Case 2 (Text-fig. 2) had a blood volume of 2,663 cc. (estimated by a method described later), or 54 per cent of total volume, shortly after hemorrhage. Case 7 (Text-fig. 7) showed a volume of 3,350 cc., or a reduction to 62 per cent some days after primary hemorrhage. It is not definitely known how low the volume can drop and the patient still live. The lowest figure in this series was 54 per cent. It is possible that lower figures might be obtained in determinations made soon after primary hemorrhage.

With the single exception of blood pressure readings, none of the other data obtained in these cases, such as hemoglobin per cent, findings with the hematocrit, etc., gave any indication of a diminished volume, and it was only after a number of observations on blood volume had been made that we were enabled to associate a lowered pressure with a small blood bulk. The blood pressure, however, is of assistance in judging the volume only when below a certain point. For there may be a considerable reduction of the total blood without any appreciable drop in the pressure. This is due undoubtedly to the fact, now well recognized, that the vasomotor mechanism responds within certain limits to the lessened blood bulk by producing a vasoconstriction which maintains the pressure. Just how great a diminution can occur before the blood pressure falls cannot be stated, and there would seem to be some variation with the individual case. In the present series we have had patients whose volume was reduced to 70 per cent of normal, yet a normal blood pressure was still maintained. Such patients probably had some fall in pressure immediately after hemorrhage, since a blood loss of the amount indicated, unless

[2] The text-figures with their legends will be found at the end of Paper II.

very gradual, practically always causes a drop. But the compensatory mechanism was able to restore the pressure to normal. The patients who had a reduction in volume below 70 per cent showed, with one exception, a blood pressure below normal, the decrease in blood pressure corresponding roughly with the amount of reduction in blood volume. The cases with a volume of 54 to 60 per cent of the normal showed systolic blood pressures of 70 to 80 mm. of mercury. One alone had a pressure of 90 mm. It is somewhat difficult to compare these figures, as the readings were not taken at the same intervals after hemorrhage, some being taken immediately and others after many hours or days. However, low blood pressure has been found to be associated so constantly with reduced volume that it seems fair to draw certain general inferences from these observations. We have come to feel that in hemorrhage cases when a blood pressure is below 95 mm. the blood volume is probably under 70 per cent. With a blood pressure of 80 mm. or less the volume is probably only 60 per cent or less. It is possible that a larger series of cases will show that these estimates need modification and that even more accurate inferences can be made from the blood pressure changes.

Patients suffering from acute blood loss and considerably diminished volume showed a much reduced pulse pressure, to 20 mm. or less. Other cases in which the hemorrhage had taken place more slowly or some time previously had a pulse pressure not under 30 (Text-figs. 3 and 5), although the blood volume was considerably lessened and the blood pressure lowered.

### *2. Amount of Blood Loss or Actual Degree of Anemia.*

The actual amount of 100 per cent hemoglobin blood remaining in the circulation was calculated by multiplying the hemoglobin per cent[3] by the blood volume, and dividing this figure by the normal blood volume (obtained later).[4] This gave the total per cent of hemoglobin—assuming that the patient's hemoglobin normally is

[3] Hemoglobin tests were made by the Palmer method (Palmer, W. W., *J. Biol. Chem.*, 1918, xxxiii, 119), which has been of great value in this study. We agree with Palmer that it is accurate to within at least 1 per cent, and we have often made repeated readings to ½ of 1 per cent.

[4] The normal blood volume could also be calculated from the weight.

100 per cent. Estimations made in this way showed that many of these patients have lost more blood than is commonly supposed. Two markedly anemic patients had a reduction of total hemoglobin to 16 per cent of normal (Text-figs. 4 and 5). These cases, however, both gave a history of the first hemorrhage occurring several weeks before. Case 2 (Text-fig. 2) had his initial hemorrhage only the day before and showed a reduction to 35 per cent. Case 7, with a history of only one hemorrhage, showed the same reduction. Other patients showed a reduction of 60 to 75 per cent of their total hemoglobin. How much they lose with a single hemorrhage it is not possible to estimate, since one cannot be sure that they have bled only once. The cases showing a marked reduction in total hemoglobin must have bled repeatedly. These results show that with a remarkably small amount of hemoglobin the circulation can supply enough oxygen to the tissues to keep the patient alive. It seems probable that a reduction much below 16 per cent would be incompatible with life.[5] Both the patients showing this low figure were critically ill.

### 3. *Distribution of the Blood in the Vessels.*

Numerous hemoglobin estimations made on blood from the ear and vein taken simultaneously have shown a definitely higher hemoglobin per cent on the capillary side when the blood pressure is low (Text-figs. 5, 6, and 9). The greatest difference in the two readings has been 8 points. The degree of discrepancy does not seem to parallel the extent of blood pressure drop in the different cases, since in the case showing 8 points difference between ear and vein the blood pressure was only 95 mm., while in Case 9 (Text-fig. 9) the difference was 6 points with a blood pressure of 65 mm. However, repeated readings in the same case show that the degree of discrepancy between ear and vein hemoglobins does have a relation to the blood pressure, since this discrepancy diminishes as the pressure rises. We have come

[5] This is supported by the findings of Rous and Wilson (Rous, P., and Wilson, G. W., *J. Am. Med. Assn.*, 1918, lxx, 219) in animals. They found that an abrupt reduction of the total hemoglobin to one-quarter of the original normal was well tolerated when blood volume was maintained.

to regard these readings as giving valuable evidence on the state of circulatory efficiency. A difference of 6 to 8 points between the capillary and venous blood must mean a considerable stagnation of red cells with a consequent diminished oxidation. It is apparently not necessary that a marked drop in blood pressure should occur before the capillary and vein hemoglobins begin to differ.

### *B. Progressive Changes in the Circulation after Hemorrhage.*

#### *1. Blood Volume.*

*Methods Employed for Estimating Volume Changes.* (*a*) *Vital Red.*—Repeated vital red tests were made on a number of cases (Text-figs. 1, 2, 4, and 7).

(*b*) *Gum Acacia.*—Blood volumes were also estimated from the drop in hemoglobin per cent produced by the intravenous injection of a known amount of 6 per cent gum acacia solution. Text-fig. 3 illustrates the use of this method. Case 3 had a hemoglobin of 61.5 per cent. After the injection of 580 cc. of gum acacia solution the hemoglobin fell to 52.5 per cent. This was a drop of 9 points, or 14.6 per cent of 61.5. With the addition of a colorless diluent to a colored fluid the drop in color should be in inverse proportion to the increased volume of the solution; therefore taking 61.5 per cent as 100, the formula for estimating the new volume would be

$$\begin{aligned} 100 : 14.6 &:: x : 580 \\ 14.6\ x &= 58{,}000 \\ x &= 3{,}973 \end{aligned}$$

Subtracting 580 from 3,973 gives the volume before the gum injection. Estimations made with vital red at the same time showed a volume of 3,844 cc., which would give an error of 3 per cent to the gum acacia estimations.

Blood volume estimations might be made in this way with other kinds of colorless fluids injected intravenously. However, gum acacia solution is particularly well adapted to this purpose, since it is retained in the blood stream, while the majority of other solutions are rapidly eliminated from the circulation.

(*c*) *Hemoglobin Changes.*—Blood volume changes were estimated in a third way by changes in hemoglobin per cent occurring as a re-

sult of fluid absorption into the circulation. The approximate increase in the blood bulk can be calculated in this manner only if the blood volume has been already determined by one of the two methods just mentioned. Such a starting-point is necessary. Case 7 (Text-fig. 7) shows the method by which the estimation is carried out. This patient had a drop in hemoglobin from 48.5 to 38.5 per cent occurring in the course of 24 hours, which equals a fall of 20.6 per cent of his original hemoglobin. His blood volume before the drop occurred was 3,665 cc. One uses the same formula as for the gum acacia volume estimation, except that in this instance we know the previous volume; therefore 100 − 20.6, or 79.4 is taken as the new hemoglobin per cent. Then

$$\begin{aligned} 100 : 79.4 :: x : 3{,}665 \\ 79.4\,x &= 366{,}500 \\ x &= 4{,}617 \end{aligned}$$

A second vital red estimation done at this time gave a volume of 4,715 cc. This difference of 100 cc. can be accounted for to a certain extent by the increased production of erythrocytes as shown by the presence of 8 per cent reticulated cells. Even though the error were several times as great as this—and it may be said that the calculation did not work out so closely as the above in all cases—the method is still of value.

*Effect of Transfusion and the Injection of Gum Acacia.*—In the present series of cases transfusions were given with both citrated blood and whole blood (paraffin tube). The blood volume was increased by both procedures, and the increase persisted equally well following either method. The effect of gum acacia injections was entirely similar in this respect. However, gum acacia solution was not used in very anemic patients. The most interesting fact revealed by the repeated blood volume tests was that transfusion or infusion only begins the work of restoration of the blood bulk. Case 2 (Text-fig. 2) is a good example. The estimated initial volume in this case was 2,660 cc., or 54 per cent of the normal. Transfusion of 800 cc. of blood increased the volume to 3,460 cc., which was still 1,440 cc. below the patient's normal volume. In other words, even after transfusion the volume was only 70 per cent of normal. It is obvious that in such

cases the volume must still be increased considerably before we can say that the circulation has been restored to a state of normal efficiency. The lacking fluid bulk is made up by the patient from his own body fluids. It has been found necessary in practically all cases to increase the fluid intake in order to bring this about promptly. By this means, in the patient referred to (Text-fig. 2) the volume was increased progressively to normal during the $2\frac{1}{2}$ days following transfusion. (This was indicated by the drop in hemoglobin to the lowest level reached on April 30 and was shown to be the fact by a vital red test made the next day.) This case is typical of others studied in the same way. Text-fig. 3 shows the same increase in volume after gum acacia solution injection. With the progressive increase in volume after transfusion the hemoglobin falls, which is itself an index of increasing blood bulk. Thus we have come to expect in transfusion cases a drop in hemoglobin and the volume of formed elements (hematocrit) following a preliminary rise. If this does not occur it is taken to signify that the patient's blood is not diluting as it should, or, otherwise expressed, is not receiving the expected fluid increment from the tissues; and an increased fluid intake is indicated. When the hemoglobin per cent drops and then gradually becomes stationary, the volume has probably reached a level well up toward normal, provided the fluid intake has been good. An exception to this is to be found in the cases of well marked anemia discussed later.

### *2. Relation of Blood Volume to Hemoglobin Per Cent.*

The extent to which the blood becomes diluted through a restoration of its volume after hemorrhage seems to depend within certain limits on the total amount of hemoglobin remaining. We have observed in patients with a very low hemoglobin per cent that the blood volume does not show a marked increase until the amount of hemoglobin rises, no matter how much fluid they are given. There would appear to be two forces at work in these cases—one which tends to restore the blood bulk so that the circulation has a sufficient volume to work with effectively, and the other a mechanism tending to inhibit dilution of the blood with the consequent lowering of the

hemoglobin per cent thus entailed. Until a very low per cent of hemoglobin is reached by bleeding, the former force is the stronger, and the blood dilutes to the volume needed for a good blood pressure. There seems to be a point at about 20 per cent hemoglobin where the latter force is the more effective and the blood does not dilute—which would reduce the hemoglobin below this point—even though the volume may be so small as to be barely compatible with life. Case 4 (Text-fig. 4) showed a condition of profound anemia—hemoglobin 20 per cent. A fairly large fluid intake was maintained, yet the blood showed no tendency to dilute, even though the volume was less than 68 per cent of the normal. Case 5 (Text-fig. 5) shows a first hemoglobin reading of 26.5 per cent and 30 per cent from vein and ear respectively. At this time the blood volume (estimated) was 2,605 cc., or 56 per cent of normal. This volume was evidently too small for the circulatory needs, and dilution was permitted to occur until the hemoglobin reached 23 per cent. At this point the hemoglobin remained practically stationary, the volume having increased meanwhile sufficiently to bring the blood pressure up to normal.

When this balance between the opposing forces of blood dilution and blood volume has been reached, any further increase in volume seems to be dependent on an increase in hemoglobin. In Case 4 (Text-fig. 4) it is seen that the blood volume stayed low until the 23rd; then, coincident with a well marked increase in hemoglobin—due to the tremendous bone marrow stimulation present, as shown by the great number of reticulated cells—the volume took a decided rise. The same phenomenon is seen in Case 1. Following the second transfusion on April 9, the volume remained as before at about 4,300 cc., then showed a tendency to drop off (the patient had marked sepsis at this time, and an amputation was performed). During this period the hemoglobin stayed at a level of 38 to 39 per cent. The production of new red blood cells, as indicated by the number of reticulated elements, had dropped almost to normal. On April 14 a marked stimulation of the bone marrow must have occurred, for the per cent of reticulated cells rose rapidly until it had reached a high figure. Coincident with this increase in blood production the blood volume began to increase, and by the 20th, if not sooner, the

volume had reached normal. Even though a volume estimation is lacking here, the fact that there was no rise in hemoglobin per cent in spite of the great increase in blood production would have led one to infer that the total blood bulk must be increasing.

It would seem from the observations in the present series of cases that in the absence of severe complications, as sepsis, shock, etc., this failure of the volume to return approximately to normal occurs only when the hemoglobin is below 40 to 50 per cent. In patients with more hemoglobin than this the volume increases to somewhere near normal very promptly. In one or two cases of marked sepsis the rule did not hold.

This finding indicates definitely the beneficial effect of transfusion in patients in whom there is a marked reduction in total hemoglobin. By the addition of new red cells to the circulation the organism is enabled to restore the blood volume to a much greater extent than would have been possible without transfusion. In other words, by means of transfusion we have taken the patient out of the class of limited blood dilution and put him into the class in which well marked blood dilution can occur.

### *3. Relation of Blood Pressure to Hemoglobin Per Cent.*

The cases with the most marked anemia, uncomplicated by any noteworthy sepsis, have shown an increase in blood pressure to above normal when the blood volume has been increased. This high pressure has persisted until an increase in hemoglobin occurred. The abnormally high blood pressure has been accompanied by an increased pulse rate as well. Case 4 (Text-fig. 4) is the best instance, as this was the most anemic patient of the series. As long as the hemoglobin remained at 25 per cent or under the blood pressure varied from 135 to 145 mm. with a pulse rate of 140. With a rise in hemoglobin the pressure fell to normal where it remained. The pulse rate showed a parallel drop, though it did not reach normal. Text-fig. 1 shows the same phenomenon. After the second hemorrhage on April 6, which was followed by a drop in hemoglobin per cent to 26, the pressure rose to 145 mm. and remained high until after transfusion which increased the hemoglobin to 38 per cent. The pulse

followed the general curve of the blood pressure but did not show a parallel drop, owing, no doubt, to the presence of marked sepsis at this time.

There seems reason to believe that this increase in blood pressure and pulse during the period of marked anemia is a compensatory reaction, which takes place in order to produce a more rapid blood flow and thereby a more effective utilization of the small amount of circulating hemoglobin. As might be expected, this increased blood pressure did not occur when the volume was very much reduced. The relation between blood volume and blood pressure has already been discussed.

*4. Changes in the Hemoglobin of Blood from Capillary and Vein.*

In cases showing a difference in hemoglobin per cent between the blood of capillary and vein, we have found that as the pressure rises with the increase in volume, this discrepancy diminishes progressively until the two readings finally become the same, indicating that an even redistribution of the red blood cells has been effected (Text-figs. 5, 6, and 7). Case 9 (Text-fig. 9) shows further the relation between the blood pressure and the amount of the difference between the hemoglobin of ear and vein. In this patient a secondary drop in blood pressure was accompanied by an increased separation of the two hemoglobin readings. It is interesting to note also in these cases that a considerable rise in blood pressure is necessary to bring the two readings together. On Text-fig. 6 it is seen that even after transfusion, when the blood pressure had reached 105 mm., the ear and vein hemoglobins were still 2 points apart. Later, following dilution of the blood and a rise in pressure to 110 mm., there was a separation of only 1 point between ear and vein hemoglobin, a difference which is practically negligible. In Case 7 with the blood pressure at 93 mm. there was a separation in ear and vein hemoglobins of 6 points. In this case also the two readings had not come together until the blood pressure reached 110 mm.

A diminishing difference between ear and vein hemoglobin may be taken as a decidedly favorable indication, even though there has not been much change in blood pressure, since it seems to show even

more accurately than the blood pressure the direction the case is taking. It is possible that this is a more delicate indication of a beginning increase in blood volume than is the blood pressure. A lack of such a diminishing difference, on the contrary, may be regarded as an unfavorable sign. This last was observed by Cannon and his coworkers[6] in shock cases. The hematocrit readings made on ear and vein blood have been found to parallel the hemoglobin changes.

### *5. Blood Production.*

We have made reticulated red cell counts in all these cases at frequent intervals, usually daily. The rapidity of increase and the height to which the per cent of these cells rises seem to depend largely on the degree of anemia present. In other words, the strength of the stimulus controls the degree of increase in blood production. In the markedly anemic cases the number of reticulated cells rises rapidly so that within 24 hours after hemorrhage the blood production may have increased to many times normal. Text-fig. 4, Case 4, shows, the day following a marked hemorrhage, reticulated cells at 5.2 per cent. By the next day they had increased to 15 per cent, and the rise continued until it had reached 25 per cent on the 7th day. The reason for the tremendous bone marrow stimulation may be interpreted here as the very low hemoglobin, beginning at 20 per cent. A contrast to this case is afforded by Text-fig. 3. The patient showed a moderate degree of anemia, with a hemoglobin of about 50 per cent. The reticulated cells increased much more slowly and finally reached only 8 per cent, or about one-third the highest per cent of Case 4. The stimulus in this instance was very much less.

As soon as the increase in hemoglobin occurs, the per cent of reticulated cells falls. This is shown well by Case 5. With a hemoglobin at 23 per cent the reticulated cells numbered 12 per cent. At this point the patient was transfused. The hemoglobin rose to 40 per cent, and the reticulated cells fell to approximately half their number. Text-fig. 4 also showed a rapid drop when the hemoglobin rose over 30 per cent.

[6] Cannon, W. B., Fraser, J., and Hooper, A. N., *J. Am. Med. Assn.*, 1918, lxx, 526.

There seems to be another factor which influences the production of new blood in these cases, and that is the dilution of the blood with the restoration of the volume after hemorrhage. Reticulated red cell counts were made on an earlier series of hemorrhage cases before particular attention was paid to the restitution of blood volume. In these cases no attempt was made to restore the fluid bulk of the circulation by increasing the fluid intake, and the lack of more than a slight drop at most in hemoglobin and red cell count in these patients after transfusion or after hemorrhage when not transfused, showed that the blood volume was being made up only slowly. The percentage of reticulated cells did not go much over 3 to 4 per cent in any of the patients and usually there was very little increase over normal. Further, when the increase did occur it was much slower than in the present series of cases in whom the volume was made up rapidly. Text-fig. 7, Case 7, shows the effect of volume restoration on blood production. The patient had the primary hemorrhage 1 week previous to the first observations, and at the time of these the reticulated cells were only 2 per cent. With the restoration of the blood volume the number of reticulated cells increased rapidly so that at the end of 24 hours they had reached 8 per cent. The reason for the relatively great percentage of reticulated cells in the present series of cases is not altogether clear. However, a possible explanation lies in the fact that in them the hemoglobins were lower on the average than in the first series, owing to blood dilution consequent on restoration of the blood volume. The diminished hemoglobin per cent per unit of blood may act as the needed stimulus to the bone marrow.

Text-fig. 4, Case 4, illustrates the value of reticulated red cell estimations as an aid to prognosis. On account of the presence of intestinal hemorrhage, transfusion in bulk was considered inadvisable, since it was thought possible that an increase in blood pressure might start bleeding. The patient was in a very critical condition, with a hemoglobin of 20 per cent, and repeated small transfusions were considered. The rapid rise in the number of reticulated cells, however, led us to believe that the patient might take care of the hemoglobin deficiency himself, provided no more bleeding occurred. Two small transfusions only were given. As can be seen on the chart, blood production occurred very rapidly—except for a pause

from the 21st to the 23rd due to possible fresh bleeding—and by the end of 6 days the patient had practically doubled his total hemoglobin.

*Blood Destruction.*—In order to determine whether or not increased blood destruction was occurring, spectroscopic tests for hemoglobin and urobilin were made on the urine in all the patients studied. No evidence of increased blood destruction was found in any of these cases.

*Blood Groups.*—Compatible donors were used for transfusion in every instance. With one or two exceptions the donors belonged to Blood Group IV. No reactions occurred after transfusion.

### *6. Hematocrit Tests.*

Successive hematocrit readings were made on most of the cases at frequent intervals. The initial results with the hematocrit were found to parallel the hemoglobin percentages, and the later findings showed practically corresponding variations. However, when blood production began to increase, the increase in cell volume, as shown by the hematocrit, rose above the hemoglobin; *i.e.*, the color index became less than 1. This difference became well marked during the middle period of regeneration, then tended to diminish in most instances as the hemoglobin reached 70 to 80 per cent. The patients could not be kept long enough to observe when the color index rose to 1 again. The repeated hematocrit readings served as a check on the hemoglobin per cent, but aside from indicating a low color index yielded no additional information.

### SUMMARY.

Blood volume tests made by the vital red method (Keith, Rowntree, and Geraghty) on patients after hemorrhage showed a marked reduction in the total blood bulk. Not uncommonly the blood volume was less than 60 per cent of the normal. The reduction after a certain point had been reached seemed to parallel the decrease in blood pressure. This relation of diminished blood volume to low pressure suggested a rough method of estimating blood volume from the change in blood pressure.

By means of the blood volume and the hemoglobin per cent the actual amount of blood loss was determined. Cases of severe anemia showed a loss of as much as five-sixths of their total hemoglobin.

Progressive changes in blood volume following hemorrhage were estimated in three ways: (1) repeated vital red tests; (2) calculation from changes in hemoglobin per cent produced by the injection of gum acacia; (3) calculation from changes in hemoglobin per cent following the dilution of the blood by the patient's own body fluids.

The effects of the different methods of transfusion and of injection of gum acacia on blood volume were observed. No differences were apparent. It was found that transfusion and gum injections only partially restored the blood volume. Forced fluids by mouth were found to bring about its complete restoration in a comparatively short time.

It was observed that the organism did not restore its blood volume beyond a certain point when a further increase in it would, by dilution, have brought the hemoglobin per cent to a very low figure. In such cases a further increase of the blood volume occurred only when the hemoglobin rose. In cases with a low hemoglobin per cent as the result of a restoration of the blood bulk an abnormally high blood pressure appeared, which continued until the hemoglobin per cent again increased.

Accompanying the low blood pressure seen shortly after hemorrhage was a well marked difference in hemoglobin per cent between capillary and venous blood, with a relative concentration on the capillary side. As compensation occurred and blood pressure rose this difference lessened until the two readings were identical, indicating an even redistribution of the red blood cells.

Reticulated red cell counts made in these cases showed that a marked bone marrow stimulation occurs after hemorrhage. However, except in the very anemic cases the degree of increased blood production seemed to depend largely on the restoration of the blood volume. The patients who were put on forced fluids, with consequent rapid restoration of blood volume, showed a much higher per cent of reticulated cells than those in whom no attempt was made to increase the amount of fluid in circulation.

# BLOOD VOLUME IN WOUNDED SOLDIERS.

## II. The Use of Forced Fluids by the Alimentary Tract in the Restoration of Blood Volume after Hemorrhage.

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### *Slow Rate of Blood Dilution Following Hemorrhage.*

Observations made recently by several workers have led them to infer that after hemorrhage in wounded soldiers the blood volume is restored relatively slowly. Govaerts[1] found that, following primary blood loss, the red cell count decreased progressively till about the end of the 3rd day, when the posthemorrhage anemia became fully established. He regarded this finding as indicative of a correspondingly gradual dilution of the blood. Others have drawn much the same conclusion from estimations of the hemoglobin per cent after hemorrhage, though from less complete data. We have found by direct blood volume tests that a small blood volume often persists for relatively long periods after hemorrhage. Patients who had been wounded several days before admission (at the Base) and had no secondary hemorrhage still showed a diminished blood volume. Case 3 (Text-fig. 3), wounded about 24 hours before the blood volume was determined, had a total blood bulk of only 3,265 cc. This low figure showed that there could not have been much restoration of the blood bulk since the primary hemorrhage. Case 2 (Text-fig. 2) was wounded about 36 hours previous to our observations. During

[1] Govaerts, P., *Ambulance Ocean*, 1917, i, 355.

operation he had a hemorrhage which was soon controlled and he could not have lost more than a few hundred cubic centimeters at most, yet his estimated volume at this time was 2,660 cc., or 54 per cent of normal. This means that his blood volume must have been low before the secondary hemorrhage. Other cases have shown a diminished volume at a much longer time after the primary hemorrhage. Case 7, as shown in Text-fig. 7, had, 7 days after the primary hemorrhage, a blood volume of only 3,350 cc., or 62 per cent of the normal volume. Another patient gave no history of secondary hemorrhage, yet 9 days after he was wounded the blood volume was only 3,000 cc. Again, a man wounded 6 days previous to the blood volume test showed a volume of 2,650 cc. The restoration of volume in the last three instances may have been slower than is usually the case, but at any rate it would seem that in wounded soldiers several days usually elapse after a severe hemorrhage before the blood bulk is made up.

The lack of prompt dilution of the blood after hemorrhage would seem to be due chiefly to an insufficient quantity of reserve fluid in the tissues, since it is from this reservoir that the circulation obtains new supplies of plasma. In other words, the body fluid reserves are low in wounded men, and they must be made up before the blood volume can increase. There may be other reasons why the dilution of the blood is delayed; but the striking fact is, as we have found, that if these patients are given large quantities of fluid by the alimentary tract their blood volume increases rapidly.

### *Effect of Forced Fluids on Blood Volume.*

We were first led to try the effect of forcing fluids by finding that even after transfusion the blood volume was still far below the normal and remained low for some time. One patient who had a blood volume of 2,723 cc. before transfusion showed an increase in volume to only 3,886 cc. 8 days afterwards. However, when such a patient was put on forced fluids—water by mouth, and in certain cases saline solution by rectum as well—the volume showed a surprisingly rapid increase which was maintained. Text-fig. 2 illustrates well the effect of a large fluid intake. In this case the volume did

not rise to any extent until the fluid intake had been markedly increased. During the 1st day after transfusion the intake was only 1,000 cc., with the result that the volume rose only 150 cc. The succeeding day, however, forced fluids were begun and the patient took in 2,750 cc. with a consequent marked rise in volume which was still further increased by the following day, when it reached normal. It is interesting to note that thereafter in this case although the large fluid intake was maintained for several days, the hemoglobin could not be forced below 53.5 to 55 per cent, which indicated that the volume had been restored.

Case 8 (Text-fig. 8) was put on forced fluids about 6 hours after transfusion. Unfortunately, the hemoglobin could not be obtained before or immediately after transfusion. At the time the fluids were started the hemoglobin reading was 66 per cent. During the course of the next 22 hours the patient took in a large quantity of fluid, both water by mouth and normal salt solution by rectum, and the hemoglobin per cent showed a drop of 20 points, indicating a marked increase in blood bulk. Volume tests were not done in this case.

*Fluid Intake and Output.*—We have a further indication that the fluid reserves are low in these cases from the fact that the output of urine was at first very small compared with fluid intake. In Case 8 the intake during the first 22 hours following transfusion was 4,400 cc., while the output was under 800 cc., or less than one-fifth the intake. This marked diminution of relative fluid output occurs only while the volume is increasing rapidly. As soon as the volume has been made up, the relation of intake to output returns to the normal proportion, roughly speaking of 3:2. In Case 7 (Text-fig. 7) the intake during the first 24 hours was 4,800 cc., the output only 770 cc., approximately 6 : 1. During this time the blood volume had increased from 3,350 cc. to 4,715 cc. In the following 24 hours the intake was 3,850 cc. and the output 1,800, about 2:1. The volume showed only a slight increase during this time—100 cc. The next day the output was almost as large as the intake. The volume by this time was approximately normal.

*Rapid Absorption of Fluids Given in Large Quantities.*

*In the Treatment of Hemorrhage.*—The prompt way in which fluids taken into the alimentary tract caused an increase in volume of the blood led us to wonder whether hemorrhage cases might not be treated by this means alone. Even if an increase of only a few hundred cubic centimeters of plasma could be brought about within an hour or so, this might be sufficient to tide the patient over the critical point, provided his blood pressure began to rise very soon.

The results in the first case treated in this way are shown in Text-fig. 5. This patient had three hemorrhages in 12 days. Following the third, which was not very severe, the blood pressure was somewhat below 90 mm. of mercury. When the first observations were taken the patient was on the operating table and his arm was being manipulated. The pressure at this time was 75 mm. The first hemoglobin observations were made at 7.35 p.m.: ear hemoglobin 30 per cent; vein 26.5 per cent. This difference showed a good deal of circulatory insufficiency in view of the fact that the hemoglobin per cent was at such a low figure. The systolic blood pressure was then 90 mm. Forced fluids were started an hour later. At 10 p.m. he had another small hemorrhage, which was shortly controlled but caused a drop in pressure from 95 to 80 mm. However, the pressure recovered fairly quickly and by 3 a.m. had reached 105 mm. The pulse had dropped from 140 to 115. By 9 a.m. the next morning the patient had taken 3,000 cc. of fluid, chiefly by mouth, the blood pressure had risen to 115 mm., the hemoglobin had fallen to 23 per cent, and the ear and vein blood gave the same reading. The estimated volume increase during the night was 600 cc. In the course of the day the blood pressure showed a downward tendency, and the ear and vein hemoglobin became separated by 1 point. This is not, however, outside the margin of error. The patient was so anemic that in spite of a high per cent of reticulated red blood cells transfusion seemed indicated. His total hemoglobin was only 15.9 per cent of normal. The hemoglobin rose to 40 per cent after transfusion. A vital red test made just after transfusion gave a volume of 4,010 cc. Subtracting 800 cc. of fluid introduced would give him a volume of about 3,210 cc. before transfusion.

Although transfusion was finally performed, it seems evident that forcing fluids after hemorrhage tided the patient over the immediate emergency, by increasing his volume sufficiently to bring the pressure up to normal. Unfortunately the fluid output was not recorded. The patient did well for 7 to 8 days, when his arm became septic. A vital red test at this time showed the same volume as before. This condition of sepsis persisted for some time, then finally cleared up, and the hemoglobin which had remained almost stationary began to rise markedly. The final volume was 4,675 cc. It was felt afterwards that the blood pressure might have been raised more rapidly in this case had the fluids been pushed more energetically at first. In the subsequent cases larger amounts of fluids were given in the first few hours. It was found that these patients could absorb saline enemata at relatively short intervals. Continuous rectal drips were also used.

Case 7 (Text-fig. 7) illustrates the effect of a more intensive fluid administration. This patient had bled 7 days previously and was apparently doing well at the Casualty Clearing Station. When first seen on June 2, 10 to 12 hours after a long ride on an ambulance train, he had a systolic blood pressure of 93 mm., a diastolic pressure of 68 mm., a feeble pulse though not rapid, an ear hemoglobin of 56 per cent, and vein hemoglobin of 50 per cent. He showed no evidence of sepsis, and it was felt that his poor circulatory condition was due to a low blood volume rather than to any actual hemoglobin lack. At 11.10 a.m. forced fluids were started,—all the water he could drink and saline enemata of 250 cc. (8 ounces) every hour. Within half an hour he had taken 900 cc. His blood pressure had increased to 96 mm. At the end of an hour the intake was a little over 1,000 cc. The blood pressure was 98 mm. Within $1\frac{1}{2}$ hours he had taken 1,150 cc.; his systolic blood pressure had reached 106 mm. and diastolic 70 mm., an increase in pulse pressure from 25 to 36 points. The ear hemoglobin had dropped to 49 per cent and the vein to 47 per cent—a difference now of only 2 points, showing an improvement in the circulation coincident with the increase in blood pressure. 2 hours later, that is $3\frac{1}{2}$ hours from the time the fluids were started, the patient had taken in 1,664 cc., the systolic blood pressure was 110 mm., the pulse pressure was 35 mm., and the ear and vein hemo-

globin read the same—48.5 per cent. A vital red estimation made at this time showed a volume of 3,665 cc. Calculation of the increase of volume from the drop in hemoglobin gave a beginning volume of 3,350 cc. (62 per cent of normal). The average vein-ear estimation beforehand was 53 per cent, the drop was 4.5 points, or 8.5 per cent of 53 per cent, and therefore

$$100 : 91.5 : : 3{,}600 : x$$
$$x = 3{,}350$$

An operation was then performed. The blood pressure showed a marked rise under the anesthetic to 148 mm., with a subsequent drop to normal. Forced fluids were continued. By 7.30 a.m. the following morning the intake had reached 4,800 cc., the output was 768 cc., the hemoglobin had fallen to 39 per cent, and the reticulated cells had increased from 2.4 to 8 per cent. A second vital red estimation showed a volume of 4,715 cc. The marked deficiency in blood volume and hemoglobin which had existed at first were now strikingly apparent. The hemoglobin remained practically stationary now for 24 hours, while the blood volume showed a gradual increase which could be accounted for largely by the high per cent of new cells. The reason the volume did not increase to normal at once is probably that given in Paper I;[2] namely, the action of a certain adjusting mechanism whereby very anemic blood is not permitted to undergo further dilution after a sufficient fluid bulk has been put in circulation to give a good blood pressure. The explanation for the subsequent drop in volume from June 6 to 9 is not clear. As regeneration progressed there was a wide separation of the hematocrit and hemoglobin readings which came together again at the end of 4 weeks.

*As Indicating Degree of Blood Loss.*—It has been pointed out, in connection with the cases just described, that the degree of dilution of the blood when fluid is forced gives an index of the amount of blood lost. In all these cases, however, the initial hemoglobin per cent was moderately reduced when the patient was first seen, indicating that considerable hemorrhage had occurred, although it told us nothing as to the amount. There is another type of case in which it is impossible to tell by looking at the patient whether there has been any appreciable

[2] Robertson, O. H., and Bock, A. V., *J. Exp. Med.*, 1919, xxix, 139.

hemorrhage. The hemoglobin does not help since it is approximately normal. The blood pressure is low, but that is said to be due to shock. A case of this type is shown in Text-fig. 9. This patient had been run over by an engine; both legs were crushed. It could not be ascertained how much he had bled. His ear hemoglobin was 94 per cent, and vein hemoglobin 88 per cent; the systolic blood pressure was 65 mm. The general opinion was that he was suffering principally from shock. He was put on fluids by rectum shortly after admission, but these were not pushed energetically enough. At the end of 2 hours operation was considered indicated, and as the pressure had not risen appreciably, he was given 550 cc. of gum acacia. A double amputation was done. During this time he had received 500 cc. of saline solution by rectum—a total of 1,050 cc. of fluid. The hemoglobin was again taken and read 61 per cent vein and 63.5 per cent ear. Fluids were kept up and by 5 p.m. the following day the hemoglobin had fallen to 50 per cent with the ear and vein readings the same. During this time, about 33 hours, he had taken in 5,720 cc. of fluid and put out only 832 cc. For the first 22 hours he passed no urine. The only interpretation possible for this marked drop in hemoglobin from over 90 per cent to 50 is that the patient must have had a marked hemorrhage at the time of injury, losing almost half his total blood. Although there may have been shock in this case, and there probably was, yet his symptoms, low blood pressure, collapse, etc., could all have been due to the tremendous and rapid reduction in blood volume.

### *Indications for, and Use of Different Methods of Treatment in Hemorrhage.*

*Estimation of the Actual Condition of the Circulation.*—In attempting to determine a patient's condition after hemorrhage with a view to deciding on the most suitable form of treatment, it is of the greatest importance to think of the state of the circulation in terms of absolute blood loss. The blood volume determinations have shown that hemoglobin per cent tells one relatively little in most cases, since there may be only a moderate lowering of the hemoglobin reading, perhaps to 55 or 60 per cent, but with a volume diminished to less

than 60 per cent of normal, so that the existing anemia is really marked. Again the hemoglobin may be much reduced, say to 30 per cent; and while a patient can get along with 30 per cent hemoglobin if his blood volume is good, with a blood volume much diminished he may be in a dangerous state of anemia. It is true that blood volume tests cannot generally be made in hemorrhage cases for obvious reasons. But the information obtained from these tests performed in a number of patients has given us a conception of what actually occurs after blood loss, and from these findings we have been enabled to associate diminished volume with the changes in blood pressure described in Paper I.[2] By means of this rough method of estimating blood volume, one is able to obtain a general idea of the total blood loss. For example, with a hemoglobin of 40 per cent and a blood pressure below 80 mm., one may estimate that the total hemoglobin remaining in the circulation is only about 60 per cent of 40, or 24 per cent at most, and that the patient needs transfusion, since his oxygen-carrying capacity is very low. If the hemoglobin was 60 per cent and the blood pressure the same as above, *i.e.* 80 mm., one could estimate the total hemoglobin at about 60 per cent of 60, or approximately 36 per cent. We have shown that a patient can get along quite well with 36 per cent total hemoglobin, provided his volume is sufficient to maintain a good pressure. Thus the chief need in a case of this kind is increased blood bulk and not new red blood cells.

*Methods Employed.* (*a*) *Transfusion.*—As already pointed out, the principal indication for transfusion is the need of more red corpuscles —oxygen carriers. We have come to feel that when the total hemoglobin falls to 25 per cent the infusion of new blood is indicated. Although a patient can live with a total hemoglobin lower than this, his margin of safety is not very great. Another hemorrhage, perhaps even a small one, may be more than he can stand. The restoration of the blood volume must also be considered in this connection. We have seen that in the presence of a much diminished amount of hemoglobin, no matter how large a quantity of fluid is given, the blood volume does not show any marked increase until new hemoglobin is added to the circulation. Furthermore, such a patient is not in a proper condition to combat sepsis, since his tissues are getting only a

bare sufficiency of oxygen. Experimentally Rous and Wilson[3] found that animals made anemic acutely by bleeding did not survive when the total hemoglobin fell to one-fifth of the normal, even though the blood volume was kept at normal. On the other hand, as these investigators showed, a hemoglobin value of one-quarter of the normal was sufficient for body needs provided the blood volume was promptly restored.

(*b*) *Gum Acacia.*—With the total hemoglobin above 25 per cent the form of fluid treatment best suited to the case depends on the severity and urgency of the circulatory condition. If the condition is such that an immediate and large addition of fluid bulk to the blood is demanded, there is no question but that gum acacia fluid should be given intravenously. A falling blood pressure of 70 mm. or less, with a rapidly rising pulse and general distress will indicate this course.

(*c*) *Forcing Fluids by Alimentary Canal.*—There is a large class of hemorrhage cases in which the condition is less urgent than in those just described, yet sufficiently severe to demand prompt treatment. It is in this class of case that forced fluids by the alimentary tract seem to be of particular use. The fluids should be given as rapidly as the patient will take them—water by mouth and normal salt solution by rectum. The patients will retain at least 250 cc. (8 ounces) given by rectum every hour and when the blood loss has been marked this amount can be given every half hour. The forcing of fluids should be kept up for at least 24 hours and better for 48 to 72 hours, so that the fluid reserves are restored as well as the blood volume. This last applies to the cases treated intravenously also. It seems questionable whether non-colloidal solutions, such as salt solution, Ringer's solution, etc., given in other ways, have the same beneficial effect as fluids by the alimentary tract. Salt solution given intravenously has been definitely shown to be eliminated from the circulation very rapidly and often leaves the blood more depleted than before. Whether solutions given subcutaneously would work as well as by rectum is not known. Theoretically with a failing circulation such fluid would be absorbed only very slowly. The peculiar benefit from

[3] Rous, P., and Wilson, G. W., *J. Am. Med. Assn.*, 1918, lxx, 219.

forcing fluids by mouth and rectum is that a fairly large percentage of such fluid appears to be added to the circulation in the form of blood plasma.

We wish to emphasize again that the methods described above of estimating the total blood loss and the indications for its treatment have been applied only to cases of secondary hemorrhage or cases of primary hemorrhage seen 24 hours or more after the blood loss.

## DISCUSSION.

*Significance of Diminished Blood Volume.*—The relation of blood pressure to a markedly diminished blood volume is evident. In order to keep the circulatory system filled, a certain minimum of circulating fluid is necessary. The dangers of a low blood pressure are well recognized, and one of the most obvious procedures in medical practice in such conditions is to introduce fluid of some kind into the circulation in order to raise the blood pressure. But is raising the blood pressure to normal all that is necessary? We have shown that the blood pressure may be normal with a reduction in blood volume of 25 to 30 per cent. Unless the volume is further reduced, there may be no drop in pressure. The adaptive mechanism which enables the vascular system to adjust itself to a small blood bulk in order to maintain a normal pressure must have a limit, and without performing actual blood volume tests one cannot say in any given case how near the limit of such compensation the individual is. Furthermore, the maintenance of a normal pressure with a diminished volume depends entirely on a vasomotor mechanism which is responding well to the increased demands made by these abnormal conditions. The circulatory balance may be on the verge of profound disturbance, but as long as the patient is quiet and at rest the compensation is held. However, if an increased strain is put on a circulation in this condition, such as an anesthetic, operation, infection, or recurrence of blood loss, the vasomotor mechanism may fail and a drop in blood pressure result. We have not had an opportunity to study in detail many instances of this kind but have observed several cases in which there was a good blood pressure with a reduced volume, and in these the reaction to increased strain seemed

to be the result of the circumstances just outlined. An example of this is Case 3 (Text-fig. 3) who had a blood pressure of 105 mm. before operation and a blood volume diminished to about 70 per cent of normal. (The blood volume was not taken before operation, but there is no reason to suppose that it changed much during this brief period as there was very little blood lost except in the amputated limb. The initial volume was probably a little under 70 per cent since the final volume, though not obtained, would probably have been about 4,800 cc.) During the operative procedure the pressure fell rapidly till it reached 70 mm. At this point the patient was given 600 cc. of gum acacia. It would seem in this instance that had the blood volume been a little greater the drop in pressure might have been avoided. If these inferences are substantiated, it is obviously important to restore the blood volume beyond the degree necessary merely to raise the blood pressure.

*Possibilities in the Early Use of Forced Fluids after Primary Hemorrhage.*—All the evidence at hand goes to show that restoration of the blood volume after hemorrhage depends primarily on the content of the body in reserve fluids. When the fluid reserve is low such restoration can take place only to a very limited degree, and the result is a persistent absence of blood dilution. It is probable that after a severe hemorrhage even a normal fluid reserve cannot supply sufficient plasma to restore the blood volume, and additional fluids are needed.[4] The reason for persisting low volumes after primary hemorrhage in the battle zone seems to be due principally to two factors. In the first place, the soldier in the trenches usually has a minimum to drink, and during an engagement he may go for several days with a very small fluid intake. The result is that his fluid reserves become much diminished. A hemorrhage at this time makes a demand for a new supply of fluid for the circulation which cannot be met, or at least met only inadequately. In the second place, after hemorrhage has taken place, no attempt is made as a rule to increase the patient's

[4] Rous and Wilson showed experimentally that after repeated hemorrhage, amounting in all to about half the animal's blood bulk, the tissue fluid reserves were so depleted that the animal could no longer restore its volume. But far greater hemorrhages were withstood when the blood volume was promptly made up.

fluids. He is thirsty and may get a certain amount of water, but often in a rush he gets comparatively little attention, as his great need for increased fluid is not realized. A further factor which undoubtedly tends to diminish the reserve fluid of the body is the profuse sweating so frequently seen in patients suffering from severe hemorrhage.

It is felt by the writers that much can be done in the treatment of primary hemorrhage by the early use of fluids as outlined above. There is no reason to believe that the circulation will not absorb fluids from the alimentary tract after primary hemorrhage, in the manner demonstrated after secondary hemorrhage. Water by mouth and salt solution (possibly even water) by rectum could be started at advanced surgical stations and continued at succeeding stations. The patients might even be supplied with water to drink during the ambulance and train journey, every effort being made to give them as much as possible. It seems probable that if these measures were thoroughly carried out in patients with hemorrhage much so called shock might be avoided, since the long journey from the advanced surgical units to the Casualty Clearing Station must put a very great strain on a circulation hampered by a low blood volume. Also in patients with a tendency to hemorrhage the importance of having the tissues well charged with fluid is evident.

We do not mean to convey the impression that we are in any way advocating forced fluids as a substitute for either gum acacia or blood transfusion. The unique value of these forms of intravenous treatment in appropriate cases is unquestioned. It is as an additional measure that an abundant fluid intake is suggested. Since fluids by the alimentary tract are so easily given, many cases of hemorrhage that cannot be transfused or infused might be aided in this way. Furthermore, in cases of moderate hemorrhage, at least, many transfusions and infusions might be saved if the restoration of the blood volume were begun promptly.

## SUMMARY.

Blood volume tests made on a number of soldiers recovering from hemorrhage have shown that in many instances dilution of the blood occurs very slowly. The principal reasons for this seem to be

(*a*) an initial lack of reserve fluid of the tissues, and (*b*) the absence of any subsequent attempt by the body to make up this fluid deficiency.

By putting such patients on a large fluid intake by mouth and rectum it has been found that their blood volume can be promptly and greatly increased. Hemorrhage cases transfused, yet still showing a low blood volume, were first treated in this way. Then the effect of forced fluids without transfusion was tried. Immediately after a hemorrhage, or as soon as the patient came under observation, he was given large quantities of water by mouth, and salt solution by rectum. Under such treatment the blood pressure soon began to show a progressive rise, the volume increased, and the red cells became more evenly redistributed, as shown by the relative hemoglobin percentages of the capillary and venous blood. These changes were often well marked after only 2 or 3 hours of the treatment.

More than this, forcing fluids in cases where the amount of bleeding is difficult to estimate on account of the presence of a high hemoglobin percentage is of distinct value, since the dilution of the blood which results serves to show the extent of the hemorrhage through the drop in hemoglobin that it entails.

In attempting to determine the condition of the patient after hemorrhage with a view to deciding the most suitable form of treatment, it is of much importance to learn the total blood loss—which is often not even indicated by the hemoglobin concentration of the remaining blood. With a total hemoglobin reduced to 25 per cent or under transfusion is needful. New blood is necessary, not only to supply more oxygen-carrying cells, but also because it actually enables the circulation to increase its volume. For, as has been pointed out in Paper I, the hemoglobin percentage must be above a certain point if a rapid restoration of the blood volume by means of the organism's own activities is to come about. With the total hemoglobin above 25 per cent the chief need is for increased blood volume, and if the patient's condition demands an immediate and large addition of circulating fluid, gum acacia solution should be given. If the condition is not so urgent, forced fluids by the alimentary tract are indicated.

The blood volume can be considerably reduced and yet a normal blood pressure maintained. It is pointed out that the vasomotor

mechanism which has adapted itself to the diminished blood bulk may in any individual case be very near the margin of its compensatory power. Increased strain in such instances may cause a failure of this mechanism with a resulting fall in blood pressure.

The beneficial results of forced fluids after secondary hemorrhage suggest the value of the early use of fluids by the alimentary tract in cases of primary hemorrhage.

We wish to express our appreciation of the help given in this work by Lieutenant Colonel Roger I. Lee, Medical Corps, U. S. Army, through whom the studies were made possible. We are greatly indebted to him for many valuable suggestions and advice concerning certain directions the work has taken. We acknowledge with pleasure the aid given by Major Binney, Medical Corps, U. S. Army, and his staff of surgeons. We are indebted to The Rockefeller Institute for Medical Research for a considerable amount of apparatus. We also wish to thank Colonel T. R. Elliott for his interest and for apparatus obtained through him from the Medical Research Committee.

## EXPLANATION OF TEXT-FIGURES.

TEXT-FIG. 1. Case 1, No. 241,145, age 23 years. Wounded Mar. 3, 1918. Penetrating wound, right thigh, lower third. No history of hemorrhage. Admitted to No. 13 General Hospital Mar. 29. Wound septic. Three hemorrhages during following 9 days; transfusions given after each. Observations begun after second hemorrhage. Developed streptococcus septicemia. Apr. 12. Amputation, right thigh. Sepsis of stump. Slow convalescence. Good recovery.

Shortly after hemorrhage the blood volume (vital red test) was found to be 3,060 cc., systolic blood pressure 80 mm., hemoglobin 42 per cent, hematocrit 17. Immediately after transfusion there was a well marked rise in blood volume, blood pressure, and number of red blood cells. The blood volume continued to increase, while the number of blood cells (red count and hematocrit) fell sharply, indicating blood dilution. A blood volume estimation on the 4th day showed a normal figure. A third hemorrhage occurred at this point, following which the hemoglobin fell to 26 per cent, and the hematocrit to 12. After a third transfusion the hemoglobin rose to 38 per cent, then remained about stationary. The blood volume subsequently fell somewhat. The number of reticulated red cells dropped to almost normal, in spite of the fact that the patient had a well marked anemia (total hemoglobin 31 per cent). This condition persisted until

after the amputation of a gangrenous lower leg, when the number of reticulated cells began to increase rapidly. Accompanying the bone marrow stimulation was a progressive rise in blood volume to normal. The hemoglobin per cent remained stationary until the volume had been restored; the total hemoglobin, however, during this time showed a marked increase.

TEXT-FIG. 2. Case 2, No. 5,442, age 20 years. Wounded Apr. 26, 1918. Severely wounded in thigh (left). Admitted to No. 13 General Hospital Apr. 27. Brisk hemorrhage during operation controlled by ligature of superficial femoral artery. Immediate transfusion of 800 cc. Severe streptococcus infection followed by good recovery.

Immediately after hemorrhage the estimated blood volume was 2,660 cc., the systolic blood pressure 72 mm., the hemoglobin per cent 64, and the total hemoglobin 35 per cent. Transfusion produced a marked rise in blood pressure, the hemoglobin rose to 72 per cent, and the total hemoglobin to 53. A fall in blood pressure occurred 2 hours after transfusion, but was followed by a rise to normal. 12 hours later a vital red test was made, which showed a volume of 3,615 cc. During this time the hemoglobin had dropped from 72 to 68. This would indicate that the volume immediately after transfusion was 3,460 cc. (see formula in text, page 145, for estimating blood volume from hemoglobin changes). The initial volume was obtained by subtracting the amount of blood transfused from this figure. With the much increased fluid intake (begun Apr. 29) the volume increased rapidly to normal and the hemoglobin fell correspondingly. (See page 156.)

TEXT-FIG. 3. Case 3, No. 42,612, age 18 years. Wounded Apr. 17, 1918. Compound comminuted fracture, femur (right), extending into knee joint. Admitted to No. 13 General Hospital Apr. 18 with history of hemorrhage. Amputation through fracture. 580 cc. of gum acacia 6 per cent infused at close of operation. Wound healed without sepsis. Transferred to England May 9 in good condition.

Observations were begun 24 hours after hemorrhage. During operation the blood pressure fell rapidly from 105 to 70 mm. At the end of a short operation the blood volume was 3,265 cc., hemoglobin 61.5 per cent, hematocrit 24. The injection of 580 cc. of 6 per cent gum acacia solution caused a marked rise in blood pressure, and a drop in hemoglobin to 53.5 per cent. The next morning the hemoglobin per cent was the same. A second vital red test at that time showed a volume of 3,844 cc. An estimation of the blood volume from the drop in hemoglobin per cent produced by the injection of 580 cc. of gum acacia gave a volume of 3,925 cc. This agreed fairly closely with the vital red test. With increased fluid intake the volume showed a further rise, and the hemoglobin fell to 45 per cent. After this point the hemoglobin per cent rose progressively. Unfortunately another blood volume test was not made.

TEXT-FIG. 4. Case 4, No. 979, age 25 years. Wounded Apr. 23, 1918. Bullet wound through abdomen. Operated at Casualty Clearing Station 10 hours later. Four perforations of colon, nine of small intestine, two of mesentery. Fecal fistula. Admitted to No. 13 General Hospital May 12. Poor condition. Severe intestinal hemorrhage May 18. Observations begun the following day. Two small transfusions on the 19th and 20th. Satisfactory improvement until development of lobar pneumonia (Pneumococcus Type III) June 4. Died June 10.

Observations were begun 16 to 18 hours after hemorrhage; blood pressure 110 mm., hemoglobin per cent 20, hematocrit 9, reticulated red cells 5.4 per cent. The following day the hemoglobin was 24 per cent, blood volume 2,900 cc., and total hemoglobin 15 per cent. The number of reticulated cells increased rapidly. For 2 days the blood volume and the total hemoglobin rose. Then a drop occurred in both these values, due probably to recurrent bleeding. However, with the tremendous increase in blood production (reticulated cells 25 per cent) the total hemoglobin began to rise again sharply, and at the same time the blood volume increased. Within 3 days the total hemoglobin had been almost doubled. As the need for new blood decreased the percentage of reticulated red cells fell rapidly. An abnormally high blood pressure persisted during the period of most marked anemia. The course of restoration of the circulation was unfortunately interfered with by the onset of pneumonia. (See pages 147 and 148.)

TEXT-FIG. 5. Case 5, No. 862, age 27 years. Wounded Apr. 10, 1918. Severely wounded in arm (right). Admitted to No. 13 General Hospital Apr. 20 from Ambulance Transport because of hemorrhage. Wound septic. Hemorrhages Apr. 21 and May 2. Transfusion May 3. Prolonged sepsis, streptococcus. Transferred to England June 23 in good condition. This case is described fully in the text, page 158.

TEXT-FIG. 6. Case 6, No. 6,383, age 24 years. Wounded Apr. 17, 1918. Severely wounded in shoulder and neck. Hemorrhage from wound of neck before admission to Casualty Clearing Station. Admitted to No. 13 General Hospital Apr. 18. Repeated small hemorrhages. Transfused Apr. 29. Operation—ligation of transverse cervical artery and vein and subclavian vein for arteriovenous aneurism. Transfused after operation. Good recovery.

This chart is included in the series for the purpose of showing the discrepancy between the hemoglobin estimations on capillary and venous blood in the presence of lowered blood pressure. When the first observations were made—some hours after hemorrhage—the hemoglobin in blood from the ear (marked *E* in the chart) was 48 per cent while that in the venous blood was 40 per cent. With a rise in blood pressure following transfusion this discrepancy diminished and the two readings finally became the same when the pressure reached 110 mm.

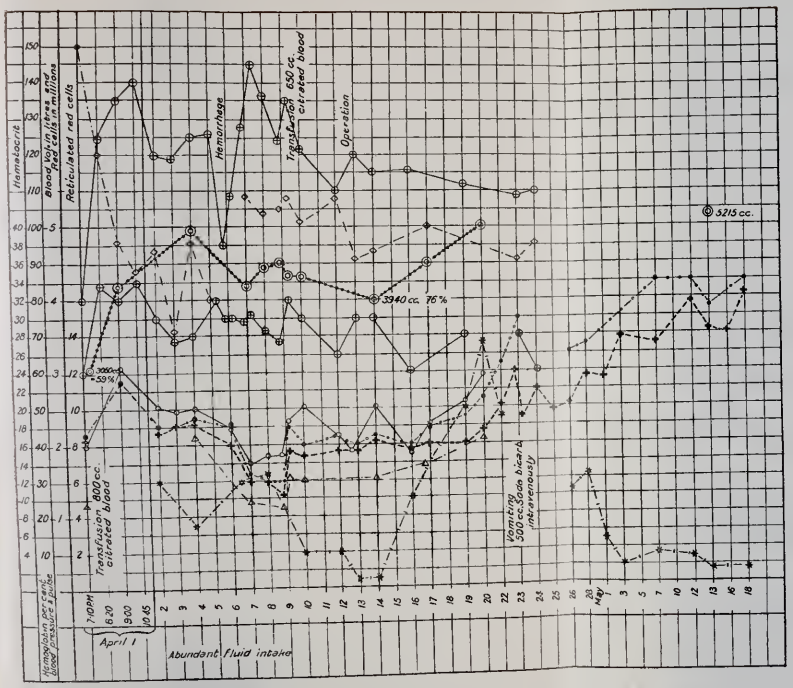
TEXT-FIG. 7. Case 7, No. 21,160, age 22 years. Wounded May 22, 1918. Multiple wounds, including compound comminuted fracture of fibula (left). At Casualty Clearing Station ligature of posterior tibial artery and vein (left). Admitted to No. 13 General Hospital May 30. No positive history of bleeding but evidence of previous hemorrhage in low blood pressure and blood findings. Treated by forced fluids. Good recovery.

This case is described fully in the text, page 159. The chart shows that the initial blood volume was only 62 per cent, but when treatment with forced fluids was instituted it rapidly increased to the normal. During this period of recovery there was marked fluid retention.

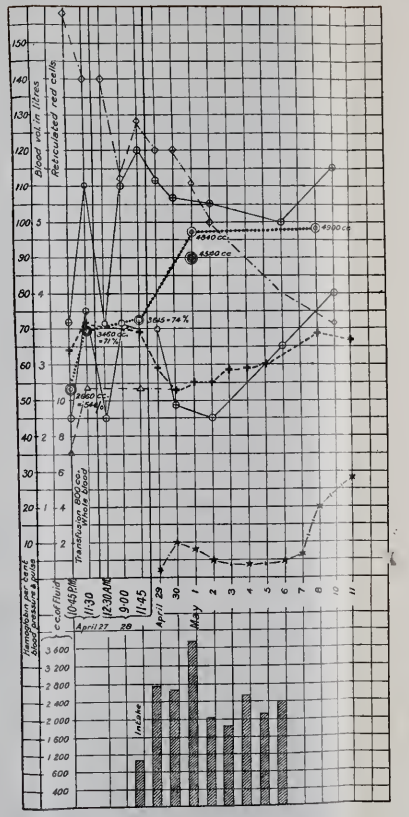
TEXT-FIG. 8. Case 8, No. 53,327, age 39 years. Wounded Apr. 29, 1918. Entrance wound below knee (right). Rifle bullet removed from groin. Fracture of lower third of femur. Moderate sepsis but wound apparently clean May 8. Uneventful course until May 12, when he had two hemorrhages. Femoral artery tied after transfusion of 450 cc. of blood and infusion of 800 cc. of saline solution. Died of gas bacillus infection May 14, 1918.

Immediately after transfusion the systolic blood pressure was 50 mm., diastolic 35 mm.; there was a well marked rise in pressure, after transfusion, to 120 mm. Observations on the blood began at this point. These are fully described on page 157.

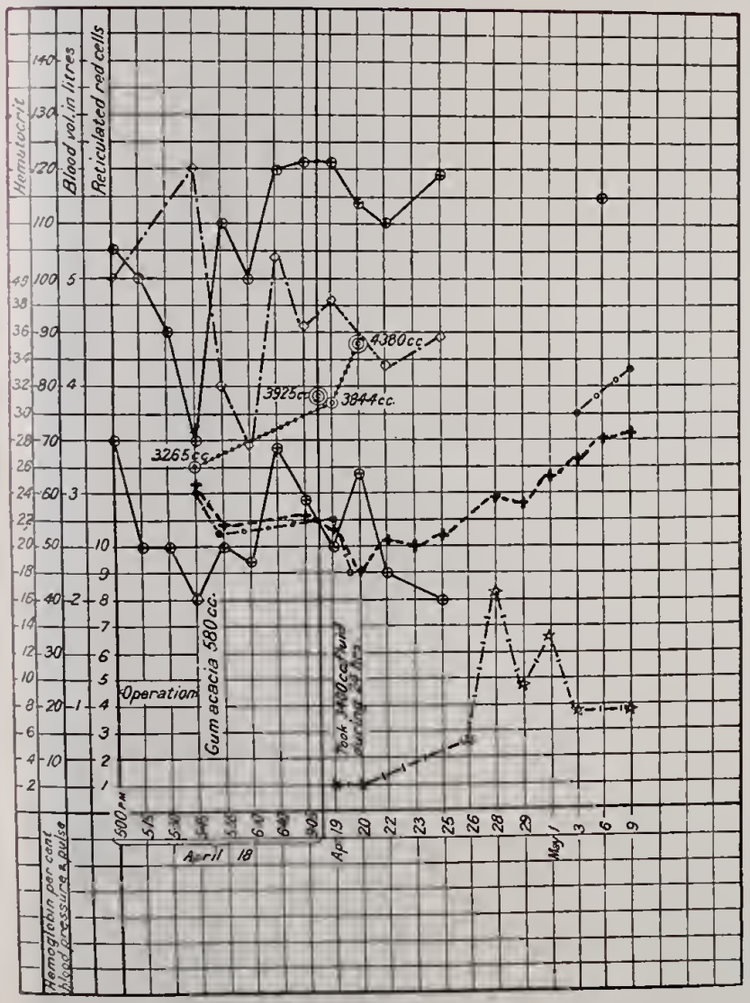
TEXT-FIG. 9. Case 9, civilian, age 29 years. Wounded June 25, 1918. Partial amputation of both lower legs by train June 25. Observations begun 1 hour later. Operation approximately 3 hours after accident. Amputation of lower third of both thighs. During operation infusion of 550 cc. of gum acacia 5 per cent. Transferred to civilian hospital June 26. Good progress. This case is described fully in the text on page 161.



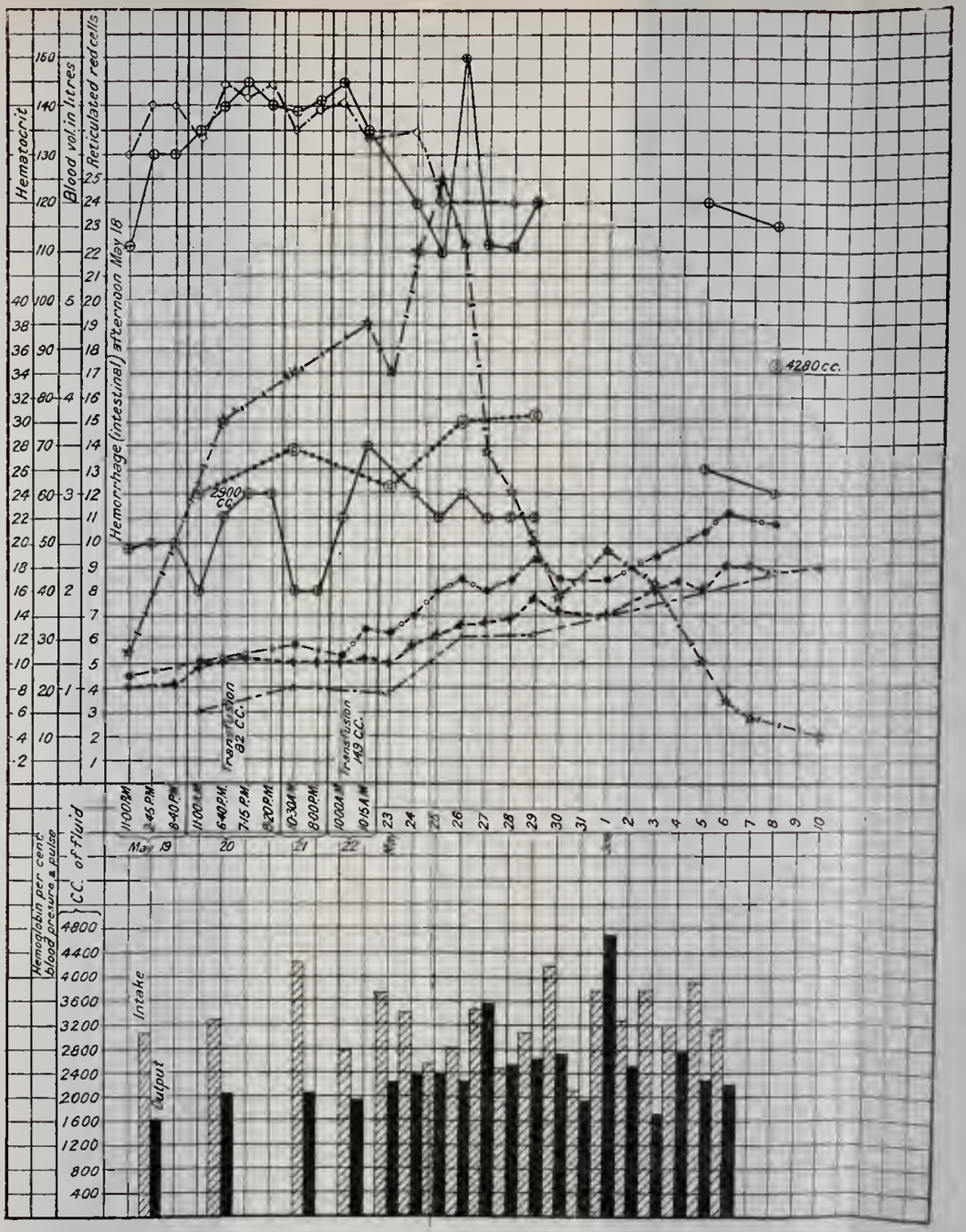

TEXT-FIG. 1. Case 1.



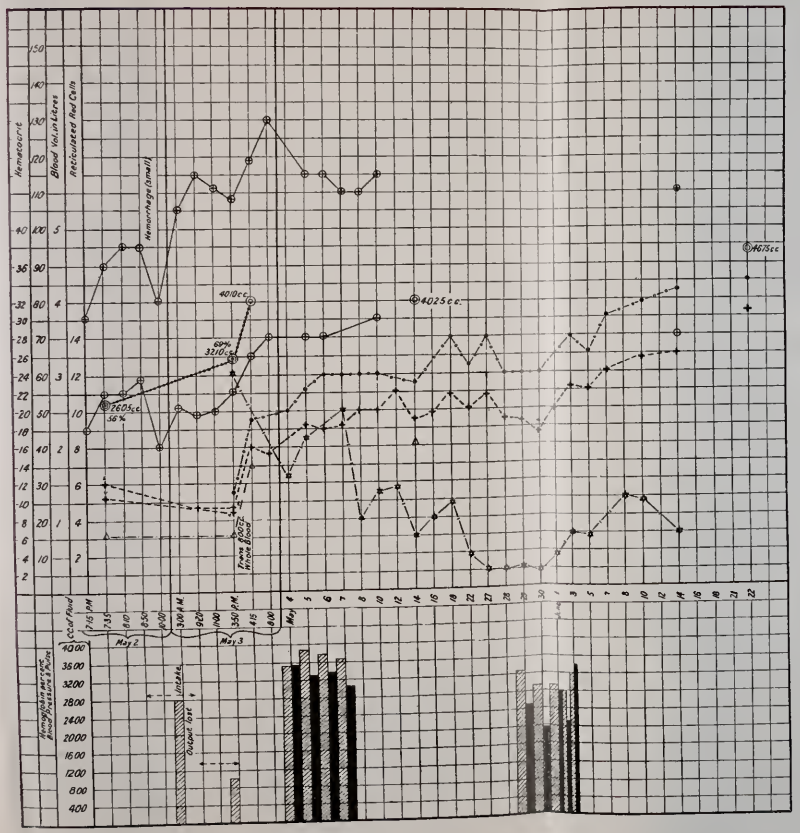

TEXT-FIG. 2. Case 2.



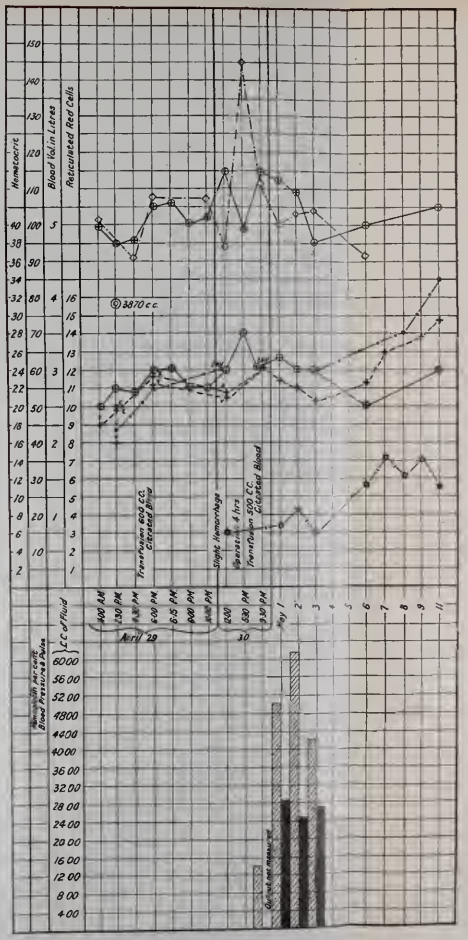

TEXT-FIG. 3. Case 3.



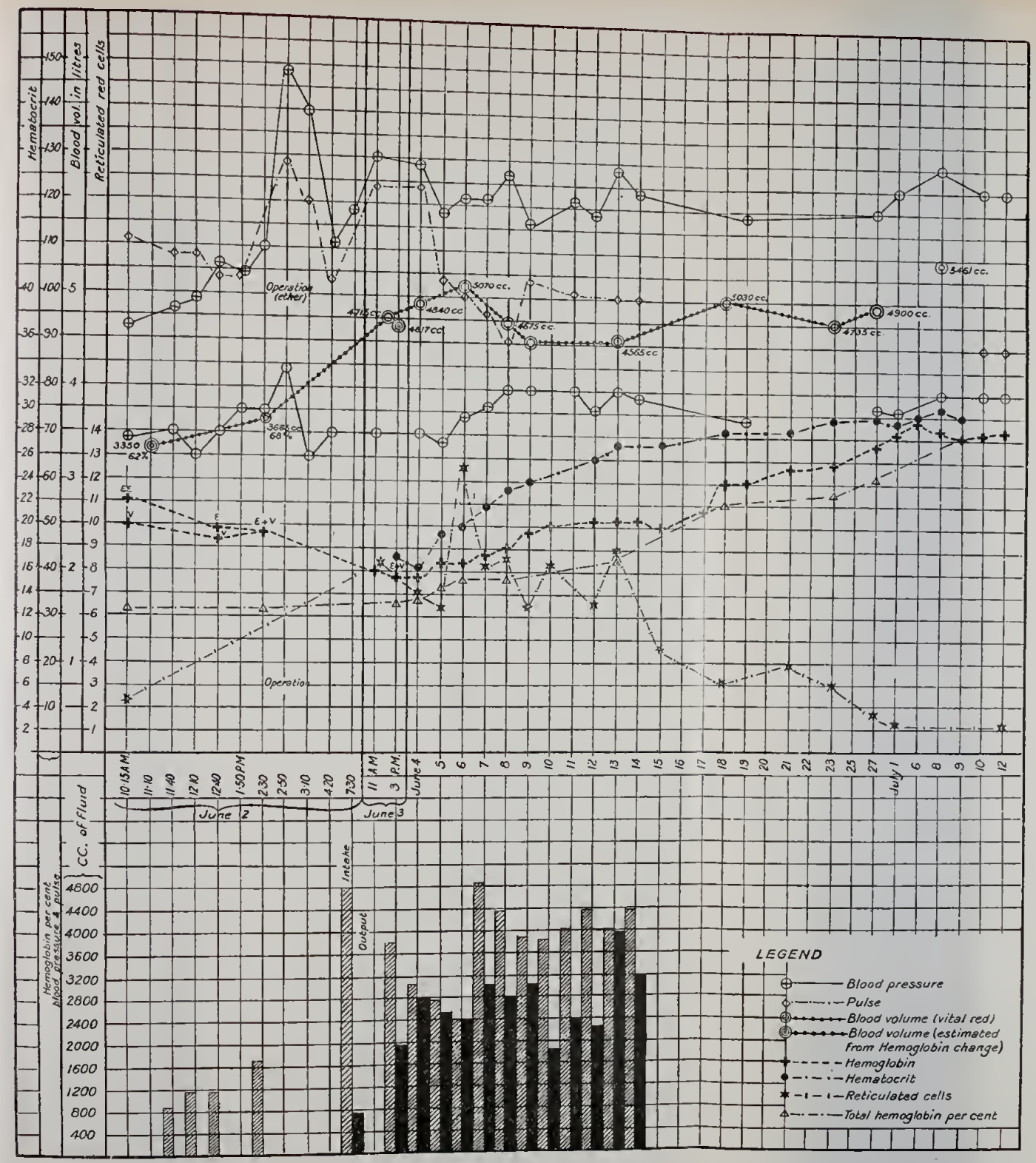

TEXT-FIG. 4. Case 4.



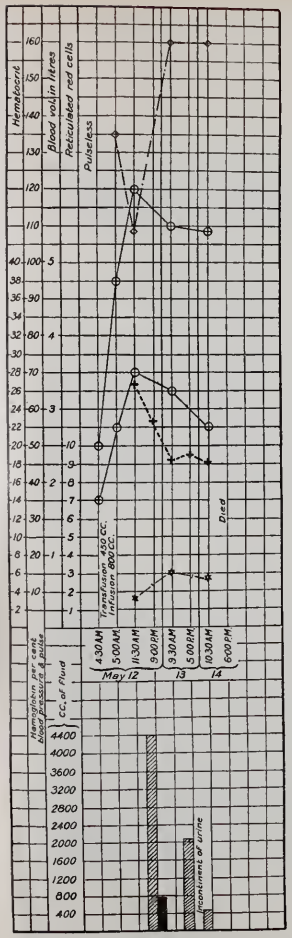

TEXT-FIG. 5. Case 5.



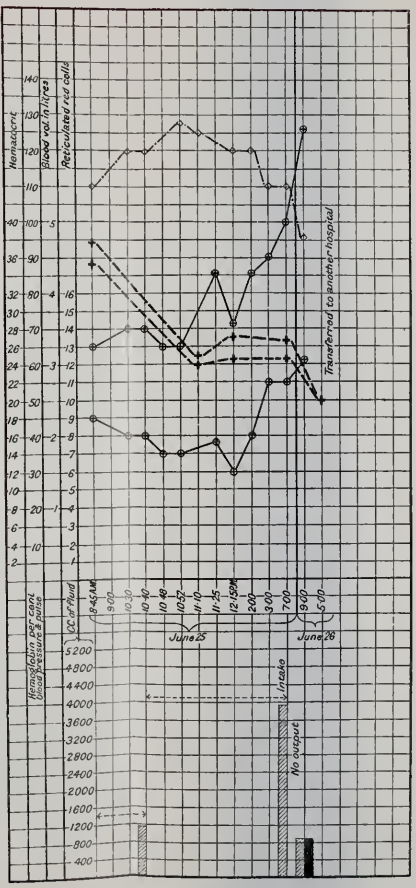

TEXT-FIG. 6. Case 6.



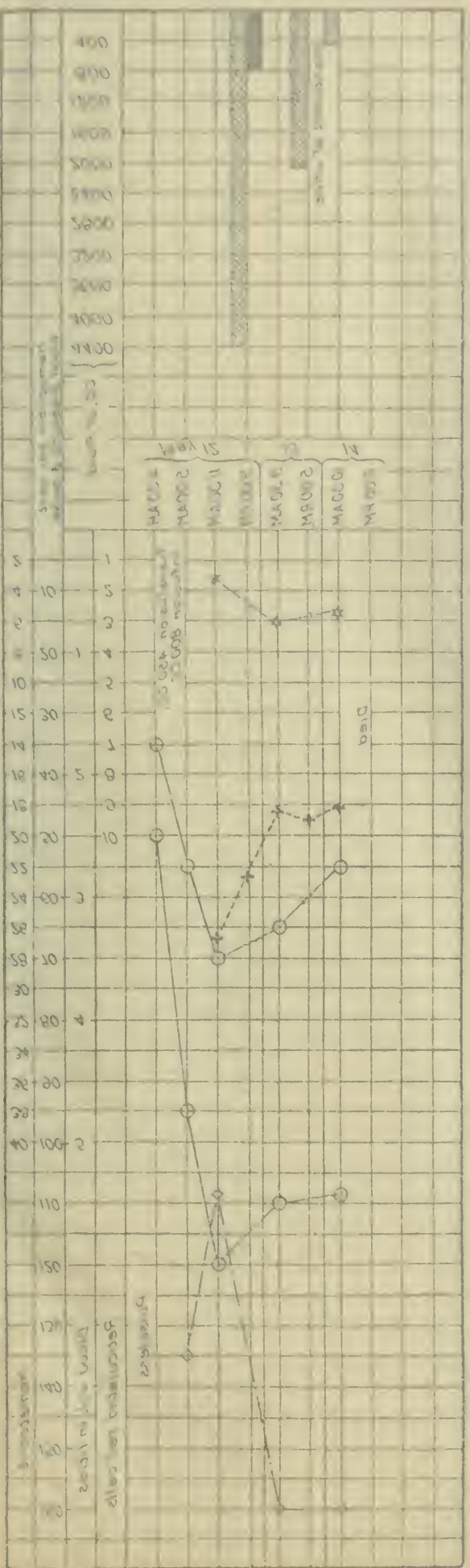

TEXT-FIG. 7. Case 7.

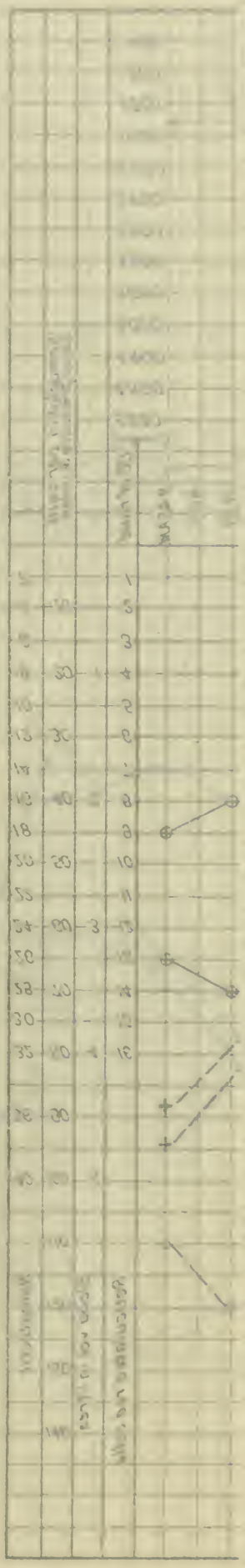



TEXT-FIG. 8. Case 8.




TEXT-FIG. 9. Case 9.





# THE INFLUENCE OF ETHER ANESTHESIA, OF HEMORRHAGE, AND OF PLETHORA FROM TRANSFUSION ON THE PRESSOR EFFECT OF MINUTE QUANTITIES OF EPINEPHRINE.

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(Received for publication, September 28, 1918.)

Epinephrine is still employed with more or less danger and success to raise the blood pressure in states of collapse. The fact lends some practical interest to the observations to be described here. Their original purpose was to determine whether the response of the blood pressure to the intravenous injection of very small amounts of epinephrine might not be a useful indicator of diminished blood volume in cases suspected of hemorrhage. In the course of the experiments ether was noted to have a marked effect on the response. And this point will be the first taken up, since it has a direct bearing on the other work.

## *General Method.*

Rabbits and dogs were used. Morphine and ether were the anesthetics given to the dogs, and paraldehyde, 1.5 cc. per kilo, was administered by stomach tube to the rabbits. A carotid artery was connected with a mercury manometer by the usual wash out cannula. The epinephrine injections were intravenous, into the external maxillary vein at its junction with the internal maxillary to form the external jugular. The flow in the jugular itself, from the internal maxillary, was utilized to flush the test doses into circulation, an important point in rabbits, since thus the introduction of a relatively considerable fluid bulk was avoided. The external maxillary ("posterior facial" in the rabbit) was dissected out for about 1.5 cm. above its junction with the internal maxillary ("anterior facial"), and a short, small cannula was introduced which carried on the end in the vessel a piece of narrow, soft rubber tubing about 0.8 cm. long. The end of the latter was pushed almost to the junction of the veins and held in place by a rubber-covered bulldog clamp, after which the sleeve of vessel wall was drawn up on the cannula and tied in place. When an injection was to be made, a hollow needle of exactly the length to reach to the end of the rubber tubing, and with a

rounded blunt tip, was thrust along the cannula, the bulldog clamp relaxed for an instant to permit its passage, and then closed on it again prior to the injection, which was practically instantaneous. In this way the test doses were delivered directly into circulation, and all injury to the vessel was prevented.

At the beginning of each experiment the minimum amount of epinephrine (Parke, Davis and Company) was determined which would bring about a well defined, if brief, rise of blood pressure, such as might conceivably be recognized in man by a coming through of the pulse beat in an arm compressed to just above the systolic pressure of the individual, as previously determined. It was found that in rabbits of 1,500 to 2,250 gm. our specimens of epinephrine caused regularly a pressure rise of 10 to 15 mm. of mercury when 0.5 cc. of a 1: 1,000,000 dilution (the commercial solution diluted 1,000 times) was injected intravenously. This will be referred to hereafter as the minimum stimulative dose. Half the amount yielded only a negligible rise in pressure. The findings were identical in ten animals. The minimum stimulative dose caused no change in heart rate or amplitude, as indicated on rapid tracings, a fortunate circumstance, since thereby factors were ruled out which might have complicated the results.

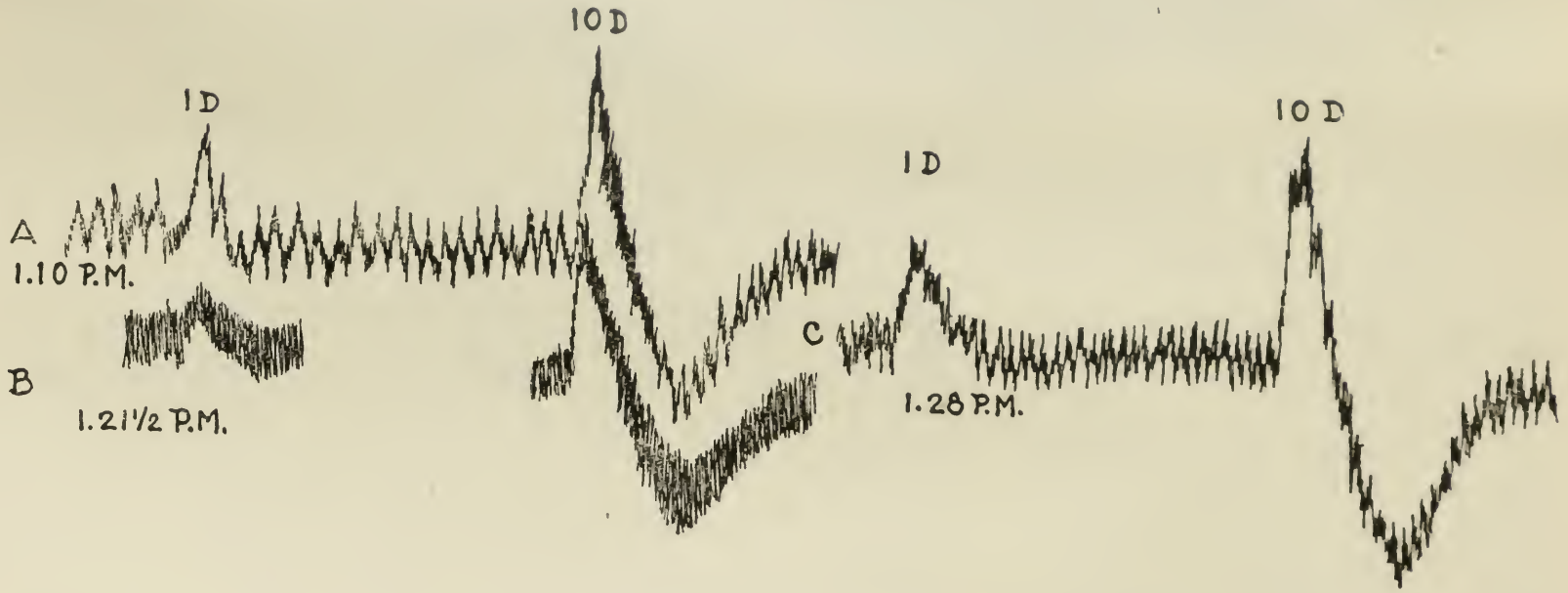
No attempt was made to determine whether the minimum stimulative dose was as constant in dogs as in rabbits.

At the beginning of each set of observations, the normal pressure response of the animal to 1 minimum stimulative dose of epinephrine and to multiples of it was several times recorded on a kymograph. The injections were given in a fixed order and at fixed intervals, and this sequence was followed throughout the experiment, in order that a possible influence of one injection to affect the response to the next might be disposed of as a disturbing element. As a matter of fact, 10 minimum stimulative doses did not suffice to alter the response to a like amount given 2 minutes later. But with 100 minimum stimulative doses it was necessary to wait more than 5 minutes if an identical response was to be obtained from a second injection. Consequently such large doses were seldom employed.

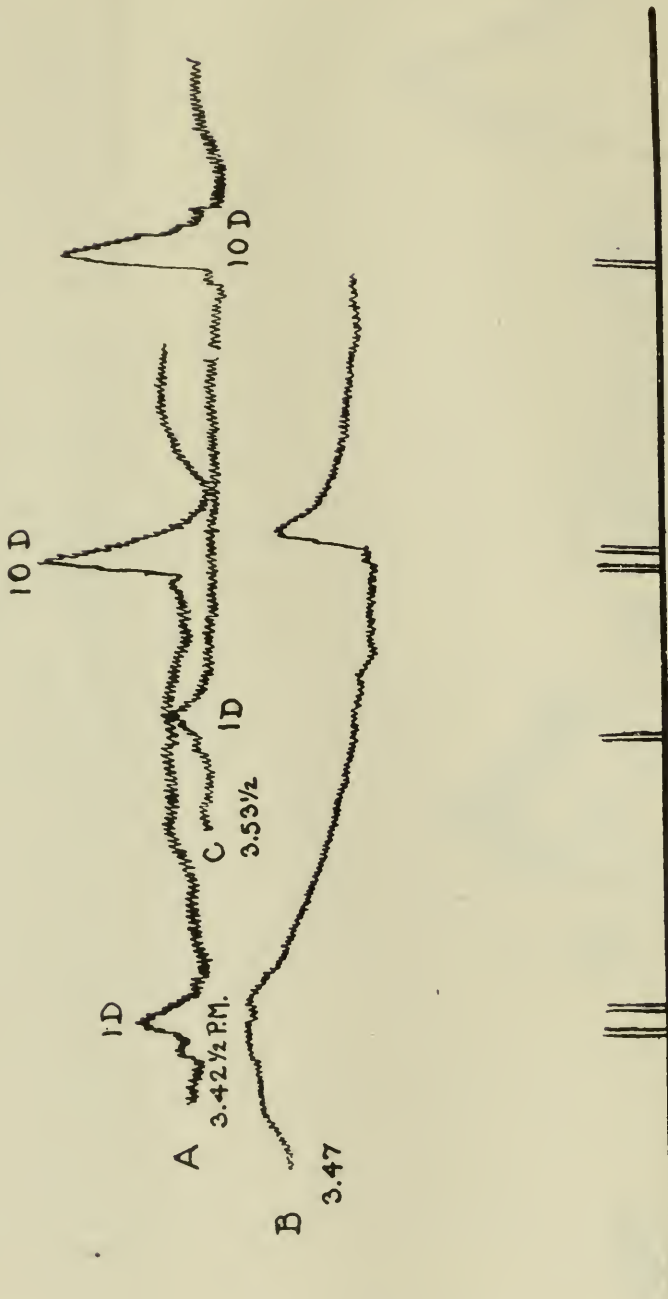
Save in special instances the fluid bulk injected at each test was 0.5 cc. Epinephrine in a dilution of 1: 1,000,000 in 0.9 per cent sodium chloride loses its pressor effect entirely in 3 to 4 hours at room temperature, and for this reason fresh dilutions had to be made in long experiments. Fortunately the 1: 100,000 dilution deteriorates little, if at all, in this time. By means of tests with this and with new 1: 1,000,000 solutions, a constant check was kept upon the results.

### *Effect of Ether.*

In rabbits under the influence of paraldehyde, and in morphinized dogs, the amount of epinephrine required to cause a transient rise in the blood pressure of 10 to 15 mm. of mercury, that is to say 1 minimum stimulative dose, was found not to vary in successive tests on the




TEXT-FIG. 1. The effect of ether on the rise of blood pressure caused by epinephrine in a morphinized dog. 1 D, 10 D, 1 minimum stimulative dose, 10 minimum stimulative doses. The fluid bulk injected at each test was 1 cc. The base-line is the same for all the records. At *A*, when the blood pressure was normal, the animal was out of ether; at *B*, 11½ minutes later, deep ether anesthesia had been produced; while at *C*, the animal was out of ether again. The dog weighed 4,750 gm. and had received 22 mg. of morphine sulfate subcutaneously about 4½ hours prior to the first observation here recorded.




TEXT-FIG. 2. The effect of ether on the response to epinephrine in a rabbit under the influence of paraldehyde. *A*, prior to ether; *B*, deep ether anesthesia; *C*, out of ether. The animal weighed 2,250 gm. and was given 2.8 cc. of paraldehyde by stomach tube 2½ hours before the first test here shown.

same animal within a period of 2 hours. When ether was given alone, or with the anesthetics already mentioned, the pressor response varied with the amount employed. Under light ether the response was similar to that with paraldehyde, but when the ether was pushed to the degree sufficient for operative procedures, 1 minimum stimulative dose of epinephrine aroused almost no response, and even that to 10 minimum stimulative doses was somewhat diminished (Text-fig. 1). When still more ether was given, so that the blood pressure fell 20 to 30 mm., the rise caused by 10 minimum stimulative doses was far less than the normal for the same animal, and, in rabbits at least, it was slow in appearing and prolonged—which doubtless accounts in part for the lessened amplitude (Text-fig. 2). The response to large doses was never affected to nearly the same degree as that to small ones.

In the deeply etherized animals there was often some cyanosis. But cyanosis alone, as brought about by a gradual stenosis of the trachea, did not affect the epinephrine response.

### *Effect of Hemorrhage.*

There is much evidence to show that the blood pressure response to small doses of epinephrine is largely conditional on the state of contraction of the vascular muscle at the moment.[1] The cells are contracted more than usual, thus narrowing the stream bed, when the blood volume is diminished after a hemorrhage. These considerations led us to test the response in bled animals with a view to its possible clinical application. For there exists at present great need of some rapid indicator of lessened blood volume. According to Cannon and his coworkers,[2] and more especially to Govaerts,[3] an approximately normal blood count may be yielded by exsanguinated soldiers seen a few hours after their wound; and it may be impossible to distinguish clinically the collapse of lessened blood volume from that due to shock alone or an extending infection.[3]

[1] Cannon, W. B., and Lyman, H., *Am. J. Physiol.*, 1912–13, xxxi, 376.

[2] Cannon, W. B., Fraser, J., and Hooper, A. N., *J. Am. Med. Assn.*, 1918, lxx, 526.

[3] Govaerts, P., *Ambulance Ocean*, 1917, i, 355.

The extensive literature upon epinephrine contains almost no work concerning the effect of small amounts of the substance on a blood pressure lowered by hemorrhage. Hoskins, Rowley, and Rosser[4] bled dogs moderately (10 to 20 cc. per kilo of body weight) and injected an equal bulk of salt solution prior to tests with epinephrine. The blood pressure was not lowered by the bleeding and injection, and in view of the fact that no preliminary steps had been taken to deprive the animal's body of fluid reserves capable of making up the blood loss, it seems probable that only slight if any adjustment on the part of the vasomotor system could have been necessary. The authors conclude that "hemorrhage causes . . . . slight or no augmentation of the reaction to epinephrine."

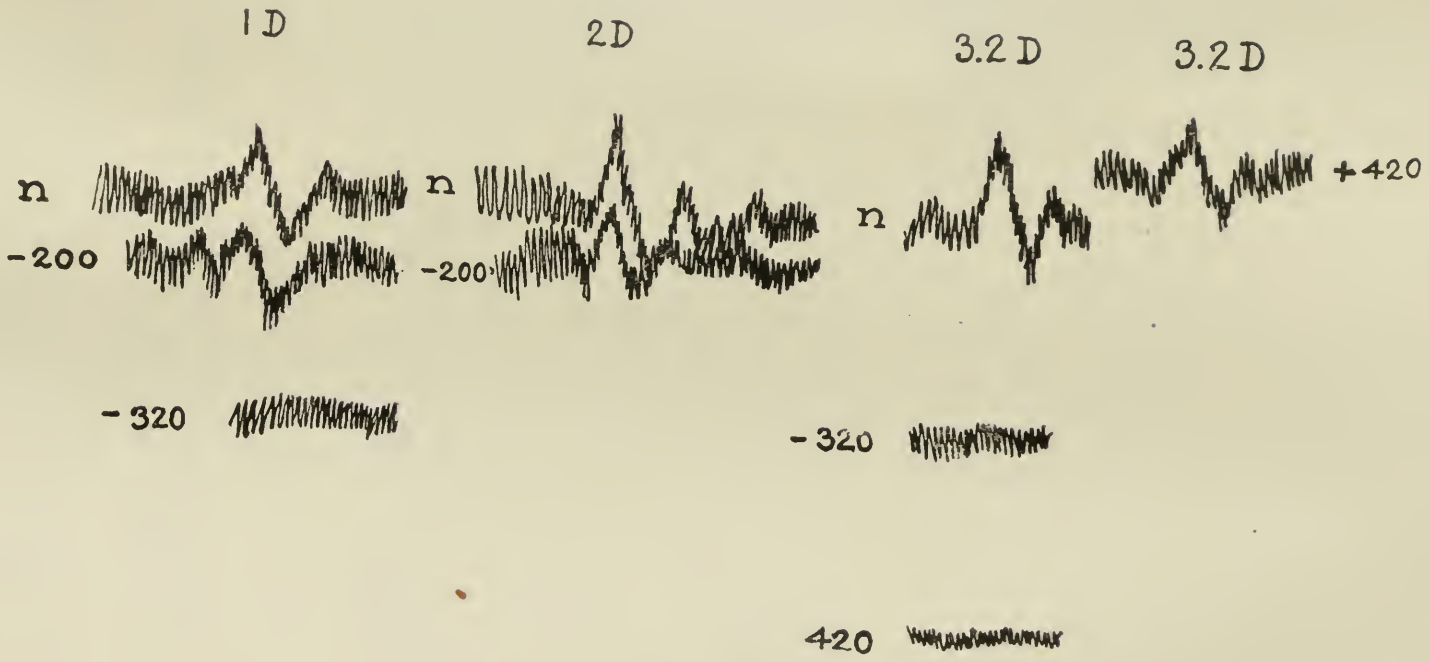
In our experiments a reduction of the fluid reserves prior to the bleedings was deemed a primary consideration. Accordingly, both food and water were withheld from the animals during the 24 to 48 hours prior to operation. Relatively small bleedings in animals so treated brought about an enduring reduction of the blood pressure, whereas in those well watered this tended to return promptly to the normal after even considerable blood losses, and the effect of the epinephrine could not certainly be judged. The bleedings were accomplished through the free limb of the carotid cannula, which latter was disconnected from the manometer temporarily and flushed of anticoagulant. In reconnecting the manometer care was taken to lower its pressure beyond that which it was thought would now exist in the circulation. Thus any considerable entrance into the blood stream of the anticoagulant—half saturated sodium sulfate—was prevented.

A marked depletion was brought about by two to four successive bleedings. After each the response to epinephrine was several times tested. Then the blood bulk was restored, sometimes with horse serum or a 7 per cent solution of gum acacia in 0.9 per cent sodium chloride, but usually by returning the animal's own warmed blood, which had been kept from clotting by 0.5 per cent of sodium citrate. When the bleedings and reinjection were carried out rapidly, the blood pressure was usually restored to its original level. But when the animal had been kept in a severely depleted condition for 20 to 30 minutes, this result was not obtained, and even when fluid was injected in excess, the pressure remained low.

Paraldehyde and morphine were the anesthetics used, and in the manner already described. To control the results, ether was sometimes employed, always under careful restriction.

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[4] Hoskins, R. G., Rowley, W. N., and Rosser, C., *Arch. Int. Med.*, 1915, xvi, 456.




TEXT-FIG. 3. The effect of hemorrhage and transfusion on the response to epinephrine of a morphinized dog. *n*, prior to bleeding; − 200, − 320, − 420, after the withdrawal of 200, 320, and 420 cc. of blood (in all); + 420 cc., after the reintroduction of the total blood removed. 1 D, 3.2 D, 1 minimum stimulative dose, 3.2 minimum stimulative doses respectively. The dog, weighing 11 kilos, received 66 mg. of morphine sulfate approximately 2½ hours before the first of the tests here shown.

The response to small doses of epinephrine was markedly affected by hemorrhages lowering blood pressure. Always a diminished pressure rise was noted, and the amount of the diminution was directly proportional to the lowering of the pressure consequent on the blood loss. When the pressure was very low, no response whatever was obtained from 1 or 2 minimum stimulative doses of epinephrine (Text-fig. 3), while sometimes 4 doses failed of effect. A response to 10 doses (0.5 cc. of a 1: 100,000 dilution) was never completely lacking, even in exsanguinated animals, but that seen was often sluggish and prolonged, as in animals heavily anesthetized with ether. That these results did not depend on mechanical inability of the cardiovascular system to raise the blood pressure, but to insufficient stimulus, was shown by the response to 100 minimum stimulative doses (0.5 cc. of a 1: 10,000 dilution), which was nearly always identical with the normal. Yet occasionally even this response was affected (Text-fig. 4).

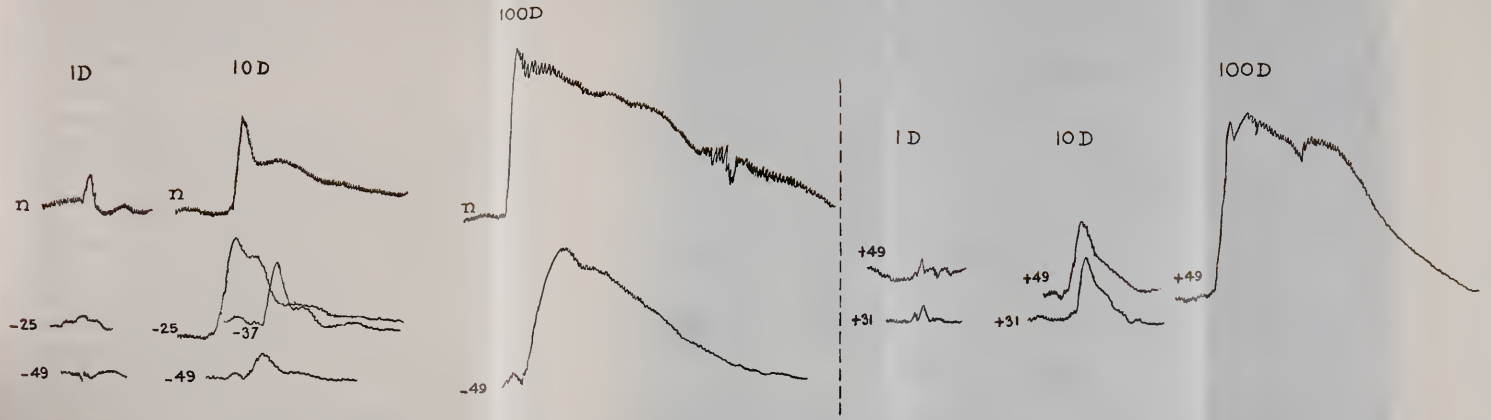
When the bleeding, testing, and reinjection of blood, or a substitute, were carried out briskly so that the blood pressure was low for only a few minutes, the response to epinephrine was not permanently impaired, but returned in proportion to the degree of restoration of the blood pressure. When the latter had reached the previous normal, the response was found to be approximately normal as well (Text-fig. 3). Prolonged depletion—for 20 minutes or more—had a lasting effect on the blood pressure which now could not be brought to the normal even by an excess of injected fluid. In such animals the response to epinephrine remained somewhat impaired (Text-fig. 4).

The results were the same in animals restored with horse serum or acacia solution instead of blood.

### *Effect of Plethora from Transfusion.*

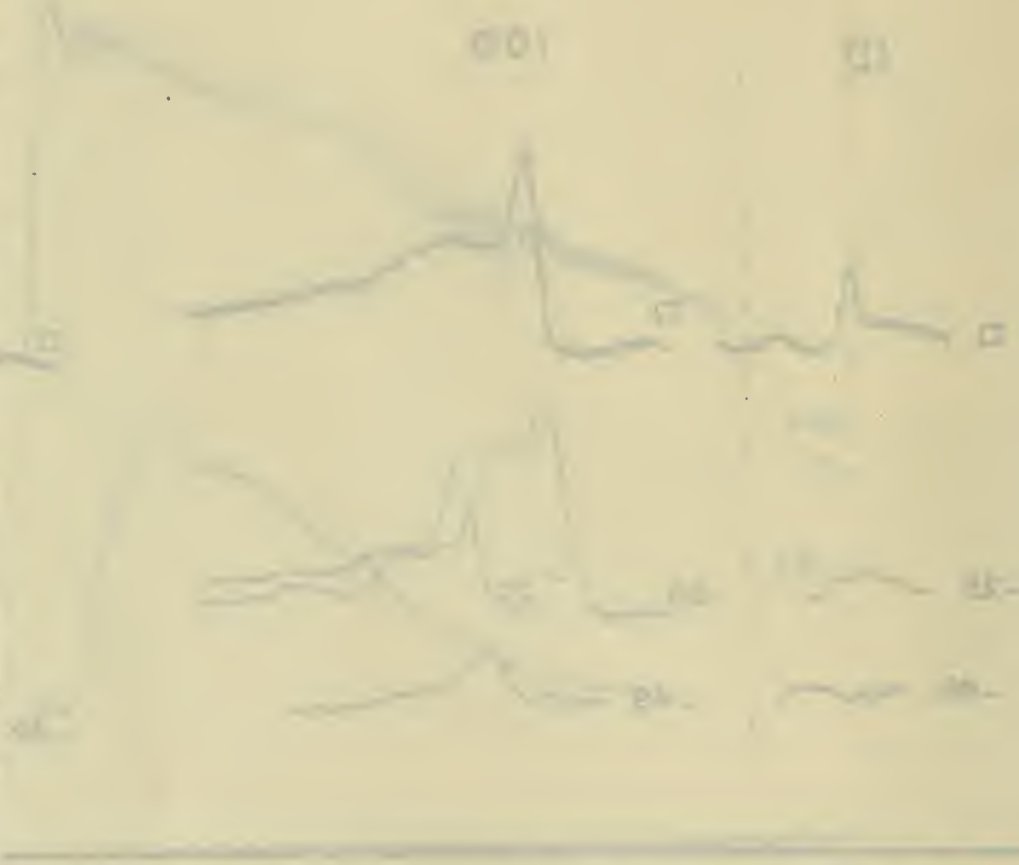
As a corollary to the findings under the condition of diminished blood bulk the influence of plethora was tested.

Rabbits were used and were given paraldehyde as in the previous experiments. A few observations were also made in animals given ether, but these were not satisfactory, owing to the great alterations in the state of anesthesia which were produced by the fluid injections. As a rule the fluid used, which was always




TEXT-FIG. 4. The effect of hemorrhage and transfusion on the response to epinephrine of a rabbit which had received paraldehyde. *n*, prior to bleeding; − 25, − 37, − 49, after the removal of 25, 37, and 49 cc. of blood (in all); + 31, + 49, after the reinjection of 31 and 49 cc. respectively of the citrated blood. The chart shows the damaging effect on blood pressure of 20 minutes exsanguination. The rabbit weighed 1,550 gm. and was given 1½ cc. of paraldehyde approximately 3 hours before the first of the tests here recorded.

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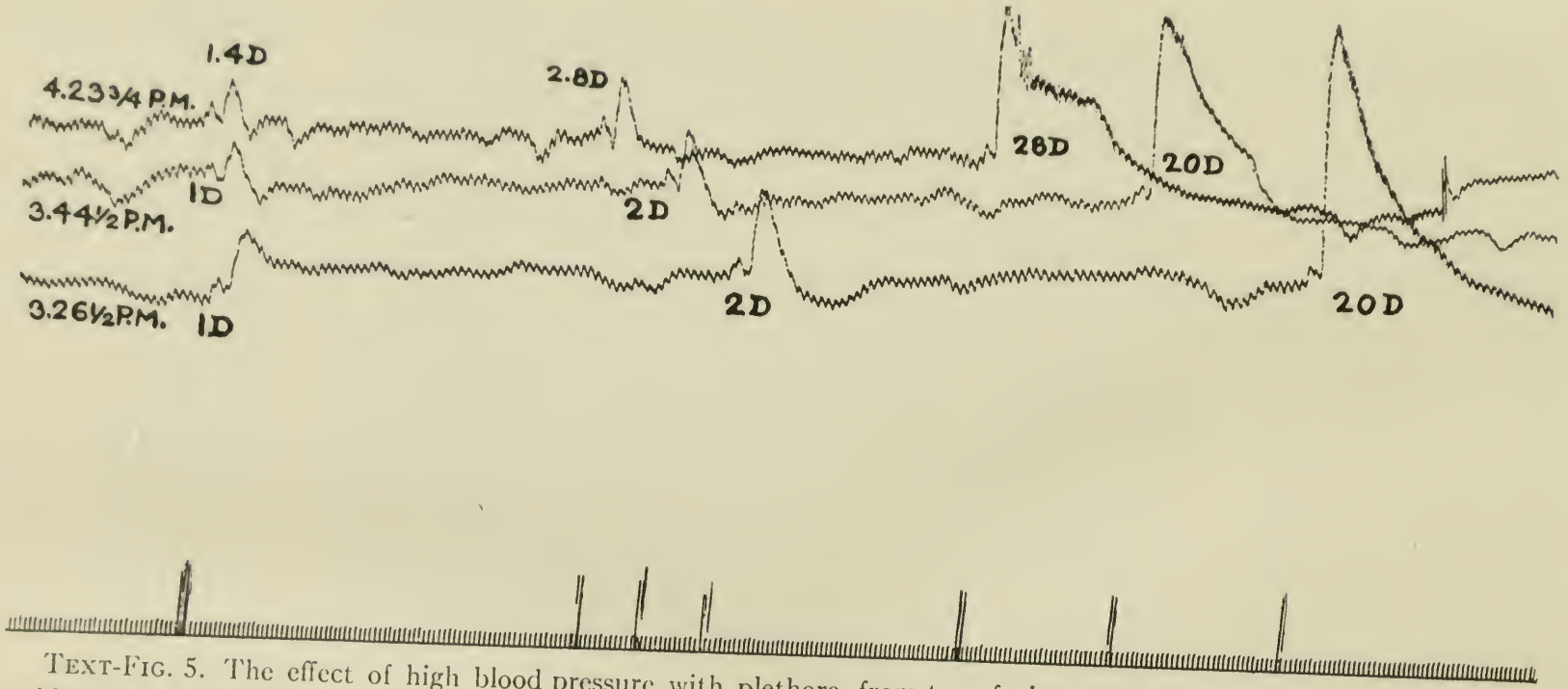
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warmed to 38–40°C., consisted of the citrated whole blood of other compatible rabbits; but sometimes a 7 per cent acacia solution was employed. Each time that the bulk of circulating fluid was increased it was necessary, of course, to increase proportionally the test doses of epinephrine in order to obtain comparable concentrations in the blood stream. For the calculations that this entailed, the original content of blood was assumed to be 5.5 cc. for every 100 gm. of the animal's crude body weight.[5] With each increase in circulating fluid produced by transfusion, the test dose of epinephrine was increased correspondingly; and it was diminished again as the fluid was withdrawn by bleeding.

The increases in blood bulk did not affect the blood pressure of the animals that were anesthetized with ether, but in those under the influence of paraldehyde every considerable increment of fluid caused a pressure rise which was permanent during the short period of the experiment. For example, an initial increase in the blood volume of about one-eleventh was usually attended by a pressure rise of 10 to 15 mm. of mercury. The observations were made then upon animals which were not only plethoric but had an abnormally high blood pressure as well. The response to epinephrine under such circumstances was regularly a diminished one; and the degree of the diminution varied directly with the heightening of the blood pressure. A diminished response was obtained even when the test dose was far greater than that necessitated by the increase in blood bulk (Text-fig. 5). When the excess of fluid was withdrawn by bleeding, the response again assumed normal proportions. The findings in plethora can scarcely be due to the sodium citrate delivered with the transfused blood, for the same results were obtained with acacia solution, and exactly opposite ones were obtained when the animals were bled prior to transfusion. However, to settle the point, the influence of citrate by itself was tested. The amounts employed in the transfusions were found not to affect the epinephrine response.

[5] Boycott, A. E., and Douglas, C. G., *J. Path. and Bacteriol.*, 1909, xiii, 256. Frequent tests of the rabbit's blood volume with vital red by a method modified from that of Keith, Rowntree, and Geraghty (Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, xvi, 547) have convinced us that this proportion closely approximates the real one.




TEXT-FIG. 5. The effect of high blood pressure with plethora from transfusion on the response to epinephrine in a rabbit. As in the preceding charts all the superimposed tracings are referable to the same base-line. The lowest tracing shows the results of tests under normal conditions; the next above the results of similar tests after the injection of 8 cc. of a 7 per cent solution of gum acacia in 0.9 per cent sodium chloride; and the highest the findings after a further 12 cc. of acacia solution. The acacia injections increased the blood volume by only $\frac{1}{11}$ and $\frac{3}{22}$ respectively of its original amount as calculated (5.5 per cent of the animal's crude body weight), yet they brought about notable rises in the blood pressure. For the tests in the normal condition and after the first acacia injection the same amounts of epinephrine were used; namely, 1, 2, and 20 minimum stimulative doses. After the second injection 1.4 times these amounts were employed, or nearly twice the increase that would have been proportional to the increase in blood bulk. The rabbit, weighing 1,600 gm., received 2 cc. of paraldehyde 3 hours prior to the first of the tests here shown.

## DISCUSSION.

It does not lie within our intent to discuss at length the physiological basis of the facts here recorded. The problems presented are obviously complex. But the findings in etherized animals cannot be passed over without a reference to the study of Auer and Meltzer[6] on the response of skeletal muscle as affected by general anesthesia with ether. These authors find that the muscle contractility is much diminished by ether, as shown both by direct stimulation and when the nerve is stimulated. They further conclude that the drug has a curare-like action, inducing a resistance to the passage of stimuli through the nerve endings. It seems probable from our work that some at least of these effects are exerted on smooth muscle. For the rise in blood pressure caused by minute doses of epinephrine is the result of contraction of the vascular smooth muscle, through stimulation at the myoneural junctions (Cushny). And when the animal is under ether the rise is much less or may be absent. One is not warranted in assuming without further work that this is the result of a diminished contractility of the vascular muscle, brought about perhaps by a block at the myoneural junctions, yet certainly it is the most likely explanation. And the effect of the ether is as transient as in the case of skeletal muscle. In proportion as the animal comes out of the anesthetic, the response to epinephrine returns.

The influence of hemorrhage on the pressure response to epinephrine may perhaps be due in part to a slowed or otherwise altered circulation of the substance in the depleted animal, so that an effective concentration fails to reach at a single moment the walls of those portions of the stream bed which are chiefly engaged in maintaining blood pressure. Whether such a factor is really active we are not enabled to say. But it will scarcely suffice to explain the lack of response to three or four times the test dose that is effective in the unbled animal. And in this connection it may be pointed out that the test dose undergoes far less dilution when injected into the circulation of an animal after the blood bulk has been decreased by severe bleeding than when injected prior to the blood loss.

[6] Auer, J., and Meltzer, S. J., *J. Pharmacol. and Exp. Therap.*, 1913–14, v, 521.

The character of the response to epinephrine when the blood pressure is lowered but the blood bulk remains intact varies with the means used to bring about the condition. If it is consequent on peptone shock or anaphylaxis, a lessened pressor response is obtained[7] for reasons as yet unknown. If, on the other hand, it has been induced by pithing or by the administration of nitroglycerol, both of which cause a relaxation of the vascular muscle, an increased pressor effect, or the substitution of one for a depressor effect, may be the result.[1] Here apparently the response is directly related to the state of the vascular muscle. Whether the lessened pressor effect observed in animals with a blood bulk decreased by hemorrhage is dependent on the unusually contracted condition of the vascular muscle—as was our *a priori* assumption—cannot be decided from the present experiments. But certainly if this is the case one is forced to conclude that only when the contraction of the muscle is very marked does any noteworthy effect obtain. For moderate bleedings which do not cause a lowering of the blood pressure, though they undoubtedly must result in some vascular contraction for its maintenance, have slight or no influence on the response to epinephrine.

The findings in plethoric animals have an interest in this connection. The repeated small transfusions by which plethora was produced would supposedly tend at some period to cause a relaxation of the vessels in order to accommodate the increased blood bulk. With such relaxed vessels an increased response to epinephrine might be expected. But as a matter of fact, any changes observed were in the direction of a lessening of the response. The marked lessening noted when the plethora was great and the blood pressure high is a new illustration of the oft noted fact that epinephrine may have a relatively slight influence on a blood pressure already above the normal (Cushny).

Neither ether anesthesia nor hemorrhage appreciably affects the pressure response to large doses of epinephrine. Herein doubtless lies the explanation for the fact that the marked influence of these factors on the response to small amounts has been overlooked. Yet this influence, as now demonstrated, is not without practical impor-

[7] Simonds, J. P., *Arch. Int. Med.*, 1916, xviii, 848.

tance. If epinephrine is to be administered to hemorrhage cases or to patients in collapse under ether, it should be given in the knowledge that doses effective under normal conditions may now fail to elicit a vascular response. If administered in considerable amount and intravenously, a temporary response, differing little if at all from the normal, can be obtained. But small amounts given subcutaneously with a view to causing a pressure rise within physiological limits will almost surely fail of effect.

It is probable that many factors besides ether anesthesia and hemorrhage will be found to lessen the pressure response to small doses of epinephrine. The statement has already been made that anaphylactic shock and peptone poisoning will do so. And we have noted that the pressure response gradually though slowly diminishes in animals given paraldehyde and kept on the operating table, with a manometer connection, for from 6 to 8 hours. From all this a successful clinical use of epinephrine as an indicator of diminished blood volume would seem unlikely.

## SUMMARY.

Ether anesthesia has a marked influence in diminishing the pressor response to minute amounts of epinephrine injected directly into the circulation. Hemorrhage also acts to lessen or abolish the response, and to a degree directly proportional to the lowering of the blood pressure it causes. In the exsanguinated animal an amount of epinephrine three or four times that sufficient to produce a pressure rise of 10 to 15 mm. of mercury under normal conditions, may be entirely without effect. The response to large doses, on the other hand, is uninfluenced by ether or hemorrhage.

The facts stated have a practical bearing not only on the employment of epinephrine to tide over collapse but on its possible utilization in the future to raise a low blood pressure to the normal height and maintain it during a considerable period. For the amount of epinephrine which under normal conditions will suffice to bring up the blood pressure may have little or no effect on an etherized individual or on one who has lost blood. The same difficulty will doubtless be encountered under other conditions.

In animals rendered plethoric by transfusion the response to small doses of epinephrine lessens in proportion as the blood pressure is increased by the plethora.

# BACILLUS EGENS. A NEW PATHOGENIC ANAEROBE.

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The bacillus described in this paper occurred in a fatal case of gas gangrene. The cultures were derived from infected muscle at a considerable distance from the wound surface, and no other anaerobes were present in the muscle. The bacillus proved markedly pathogenic for guinea pigs, producing extensive muscle lesions from which pure cultures of the bacillus were always obtained. Thus there is good proof that the bacillus found was the responsible agent in the fatal human case.

Morphologically the similarity of the bacillus to *Bacillus welchii* is marked. Its cultural reactions are such that in a mixed culture with *Bacillus welchii* it would escape notice, unless the most careful plating were done. When it has once been isolated, however, it is culturally and pathologically distinct.

*Case.*—X, age 20 years.

Day 1. Wounded. No Casualty Clearing Station note.

Day 2. Arrival at Base with multiple wounds of legs. There is a penetrating wound of the right thigh, and the whole thigh is much distended and tense. Clinically it is considered an obvious gas bacillus infection. X-ray examination shows two small foreign bodies, one in the right knee and one in the right thigh.

Day 3. A severe gas bacillus infection, extending up the abdominal wall. The thigh is blown up tightly, with gas crackles everywhere. Incision into upper thigh muscles reveals complete gas bacillus infection of the outer part of the thigh. Amputation is considered useless. 10 cc. of anti-gas bacillus serum are given intravenously in 200 cc. of saline solution, followed by 90 cc. undiluted.

Day 4. Condition is about the same, with slight increase in the extent of infection.

Day 5. 110 cc. of serum given intravenously. The gaseous emphysema now extends to the level of the costal margin, but the patient's general condition is not very bad. The thigh is still very foully gaseous. The patient vomits practically everything taken.

Day 7. Died at 1.15 a.m.

*Autopsy.*—Performed 9 hours after death. Extensive gas gangrene of the right thigh. The muscles are greatly swollen and gaseous, gangrenous in the oldest part of the lesion, showing the usual variations in hue according to the age of the lesion. There is marked friability of the muscles. There is a small amount of subcutaneous edema, and edema between muscles, with reddish fluid. Iliopsoas muscles within the abdomen pale and soft but not gaseous. *Kidneys.*—Slightly swollen and pale on section, with markings obscure. *Spleen.*—Small. Appears normal on section. *Liver.*—Acute degenerative changes. *Lungs.*—Normal. *Heart.*—Normal. *Peritoneum.*—Normal.

*Specimen Used for Cultures.*—A large piece of muscle was excised aseptically from the right thigh at operation, before treatment was started. None of the original wound surface was included in the specimen. On arrival at the laboratory the surface was seared and pieces were cut with a sterile knife from within the mass, planted in chopped meat medium, smeared on egg slants, and incubated anaerobically in McIntosh and Fildes' jars.

*Cultures.*—In 24 hours numbers of isolated colonies were present on the egg slant. All appeared alike. Several were fished into milk, but gave no reaction within 4 to 5 days. Slides of the colonies showed a bacillus resembling *Bacillus welchii*, varying more in length, however, than is usual with *Bacillus welchii* under similar conditions. Further incubation and observation of the egg slants showed no new developments. The milk test was repeated on numerous colonies, but was always negative.

The chopped meat culture gave a bacillus resembling *Bacillus welchii*, but heavily planted subcultures in milk gave no reaction in spite of good growth. Streaked on egg slants, it gave only colonies like those from the original muscle planted on egg.

Colonies from the original egg slant and from the meat subcultures were then plated on egg slants and single colonies were picked and immediately replated. The resulting colonies were always alike in gross and microscopically. After the plating had been repeated a number of times, several colonies were picked and run through media in parallel for determination of characteristics.

The above account of the cultural methods shows that *Bacillus welchii*, if present, could not have been missed, for it would have given the characteristic milk reaction in the plantings from the original

cultures. The milk used was tested by running a *Bacillus welchii* in parallel. It also proves that the original muscle contained a pure culture of the bacillus in question.

### *Characteristics of Bacillus egens.*

*Morphology.*—The bacillus is morphologically practically identical with *Bacillus welchii* when grown in most liquid media. It is of the same length, thickness, and shape. In animal tissues and in the first subcultures in bouillon the bacillus shows a well defined capsule, easily stained by gentian violet according to the Welch method. The bacillus is strongly Gram-positive in young cultures.

The differences in form between *Bacillus egens* and *Bacillus welchii* are evident in surface colonies of 2 days or more on a medium which is not alkaline. Occasionally *Bacillus welchii* produces long thread-like forms in old cultures on alkaline sugar-free media, but there is little variation in length on 0.5 per cent acid glucose agar or on the neutral egg slants. *Bacillus egens*, however, in cultures over 1 day old produces long thread-like forms on these media, often in great abundance. They are especially marked in the condensation water of egg slants and on glucose agar. These long forms are tortuous, unjointed, and Gram-positive. From forms five to ten times the length of *Bacillus welchii* they soon grow to a length extending from one to three times the diameter of an oil immersion field.

The second principal difference in appearance consists in the early appearance of Gram-negative forms of *Bacillus egens*. Often 50 per cent of the bacilli in surface colonies appear Gram-negative in 2 days. In cultures 4 to 5 days old nearly all the bacilli except some of the long threads are Gram-negative, and a great many of them are greatly degenerated, forming small, non-refractive, swollen, nearly spherical masses. *Bacillus welchii* shows few if any Gram-negative forms under corresponding conditions, and no similar degenerated forms. This tendency to Gram-negative forms is less evident in liquid media.

Another less definite difference consists in an unevenness of outline in 1 day milk cultures which *Bacillus welchii* does not show.

*Motility.*—The bacillus is absolutely non-motile in cultures, and in the infected fluids of animals. *Bacillus fallax* and *vibrion septique*

were used for a test of method, and both were found definitely motile in the medium used (condensation fluid of egg slants).

*Spores.*—No evidence was found of spore formation.

### *Cultural Characteristics.*

*Anaerobiosis.*—*Bacillus egens* is a fairly strict anaerobe. No growth could be obtained aerobically on slants, and an almost imperceptible cloud formed in the depths of a glucose bouillon tube which had been shaken with air.

*Proteolytic Action.*—No proteolytic action occurs on the casein of milk, coagulated egg albumin, cooked muscle, or coagulated horse serum, even after long exposure. This is in contrast to its softening effect on living muscle in infected animals. *Bacillus welchii* shows the same contrast in less degree.

*Saccharolytic Action.*—The only sugar fermented quickly and vigorously is glucose, in which marked acid and gas formation occurs in 24 hours. Some of the other sugars are feebly attacked. Often they show no change or a very slight one unless a heavy planting is made. Serum water media were used, and a culture of *Bacillus welchii* run as a control.

Maltose and saccharose under favorable conditions show slight acidity and gas in 24 to 48 hours. Lactose is fermented with great slowness and feebleness, never showing more than a trace of acidity to litmus. In milk not enough acid is produced to cause an acid clot in less than 12 to 14 days, even with a heavy growth.

Galactose and raffinose are fermented to the same degree as saccharose.

Mannite, dulcite, inulin, glycerol, and salicin are not changed.

*Indol Production.*—No indol is produced.

*Hemolytic Action.*—Surface and deep colonies on blood agar show a definite hemolytic zone in 24 hours.

*Odor.*—No noticeable odor was produced in any cultures.

### *Appearance of Cultures.*

*Milk.*—No gross change occurs in milk in less than 12 days, and usually not for 15 to 20 days, when a slow precipitation of casein

occurs followed by acid clot formation with little or no gas. The fluid above the clot is cloudy and gray. No digestion of the casein or further change occurs.

This absence of the stormy fermentation seen with *Bacillus welchii* is a constant and definite character of *Bacillus egens*, whether the cultures are freshly isolated from animals or have been under cultivation for 6 to 7 weeks. Tests were made with numerous cultures with heavy plantings, and good growth occurred within 24 hours. *Bacillus welchii*, run in parallel on the same medium, always produced the characteristic stormy clot.

*Chopped Meat.*—A few gas bubbles, but no other change. No digestion, blackening, or other color production.

*Egg Slants.*—No blackening or softening.

*Glucose Bouillon.*—Diffuse cloudiness with fine sediment. Diffuse cloud persists indefinitely.

*Plain Bouillon.*—Faint diffuse cloud.

### *Colonies.*

*Glucose Agar.*—In 24 hours the colonies are 0.5 to 2 mm., rather flat, circular, with a sharp even edge. By transmitted light against a black background they appear a bluish gray with scarcely greater opacity in the center. With a hand lens the colony is seen to have a very fine grayish grating in a clear translucent stratum. With the light at the proper angle reddish, purplish, and green iridescent tints are visible with a hand lens. They are probably due to refraction of the light by the fine internal structure of the colony. *Bacillus welchii* colonies under similar conditions have a large, dense, opaque, yellowish white central portion, with a translucent border filled with coarse white flecks, and show no iridescence. *Fallax* colonies are like *Bacillus welchii*, but a little less opaque and coarse.

Further incubation produces a slight increase in size and the refractive qualities decrease, but otherwise there is no special change.

Deep colonies on glucose agar are very small ($\frac{1}{3}$ mm.), opaque, white, lenticular specks without processes. They change little in size. *Bacillus welchii* produces much larger colonies and forms a larger amount of gas. On egg slants the colonies are circular with a

sharp regular outline. Within 24 hours they are pale gray but soon become a light yellow color. The surface is extremely smooth, even, and shiny. The size varies from 1 to 2 mm. at first, to 4 to 5 mm. in 2 to 3 days. *Bacillus welchii* in comparison shows after 2 to 3 days a grayish white, elevated center, with a flat, dirty yellow, slightly crenated border. Both have a surrounding zone of light discoloration of the egg medium.

Deep colonies in blood agar become brown in 2 to 3 days with a small, central, darker brown dot.

### *Pathogenic Action.*

*Bacillus egens* proved highly pathogenic for guinea pigs and somewhat less so for rabbits. After 6 weeks of subculture 0.1 cc. of a 24 hour bouillon culture was fatal to guinea pigs. After passage through guinea pigs 0.02 cc. of a 24 hour glucose bouillon culture was fatal within 30 hours. Experiments in rabbits were few, but 2 cc. of a 24 hour glucose bouillon culture was fatal to a rabbit in 2 days.

The disease in guinea pigs has the following characters.

*Clinical Characteristics.*—1. Constitutional symptoms of dullness, apathy, and sluggishness of reflexes. Fever is followed by subnormal temperature.

2. Local signs consisting in a marked soft fluctuant swelling at the site of inoculation with brownish or slightly greenish discoloration of the skin. There is local tenderness and warmth.

3. Soft sodden edema over abdomen, chest, and neck.

4. Slight gas crepitus over site of inoculation, abdomen, and chest.

*Pathological Characteristics.*—1. Extensive and complete muscle destruction in the region of inoculation. Many of the muscles are reduced to soft, pale, pulpy fragments lying in a thin red fluid. On picking up one of the pieces it falls apart by its own weight. Muscles less affected are easily torn. All the lesions are characterized by extreme pallor of the muscles. *Bacillus welchii* produces some local muscle destruction but not to such a degree, and the muscles have a different color.

2. Extensive muscle lesions distant from the point of inoculation, especially over the abdominal wall and chest. Sometimes the muscles

of the opposite leg are affected. The muscles are pale with a faint, light, purplish pink tinge, are very soft, and tear with great ease.

3. A pseudogelatinous dull red edema, usually marked in the flanks and axillæ, and often thick over the sides of the abdominal wall. It appears gelatinous to sight and touch, but on cutting it a thin, slightly brownish red fluid escapes and the immediate area entirely collapses. It therefore simply consists in a thin fluid held in very small compartments made up of thin translucent recent adhesions.

4. Gas bubbles thinly scattered in the subcutaneous tissue of abdomen and chest.

5. Absence of any odor different from that of a normal dead guinea pig.

6. No destruction of skin or loosening of hair.

7. Invasion of peritoneal cavity and blood stream.

A positive blood culture at death, or soon after in the cases where autopsy was not done immediately, was always obtained and usually bacilli could be found in blood films.

In the following protocols of experiments all these details will not be given as they are qualitatively the same in each case.

### *Technique.*

Each animal was shaved at the site of inoculation and the skin cleaned with methylated spirits followed by ether and iodine. The cultures were injected into the muscles of the thigh with a very fine, sharp needle, so that trauma of muscle was reduced to a minimum as a factor in the pathogenicity. Unless otherwise stated the cultures were 24 hour growths in 1 per cent glucose bouillon.

*Guinea Pig 1.*—2 cc. of a 2 day culture in chopped meat medium (rather thin growth). In 24 hours marked local signs of the usual character. The guinea pig appeared dull and sick. Died 36 hours after inoculation.

*Autopsy.*—Performed ½ hour post mortem. A large pocket is found at the site of inoculation filled with thin red fluid in which lie pulpy muscle fragments. Nearly all the muscles of the thigh are gone. Subcutaneous edema over abdomen and chest with a few gas bubbles. *Peritoneal Cavity.*—No fluid.

*Cultures.*—Pure cultures from the edema fluid, affected muscle, and heart's blood.

*Films.*—Films from the edema fluid show large numbers of the bacilli, which are short and resemble *B. welchii*. There are practically no cells. Blood films show a few bacilli.

*Guinea Pig 2.*—The same inoculation as Guinea Pig 1. Similar clinical course and pathological condition.

*Guinea Pig 3.*—0.3 cc. of a 24 hour glucose bouillon culture from a colony from the heart's blood of Guinea Pig 1. Died in 18 hours.

*Autopsy.*—Performed within ¾ hour after death. Lesions similar to those of Guinea Pigs 1 and 2 with a smaller local pocket of fluid. Cultures and films give similar results, with the addition of a positive culture from the peritoneal cavity.

*Guinea Pig 4.*—0.1 cc. from the original culture. (No passage through animals; under cultivation for 6 weeks.) Died in 28 to 36 hours.

*Autopsy.*—Performed 1 to 8 hours post mortem. Lesions of the usual type. Pure cultures from edema, muscle, heart, and peritoneum.

*Guinea Pig 5.*—The same inoculation as Guinea Pig 4. In 12 hours marked local swelling, tenderness, and discoloration of skin. Guinea pig sluggish and sick. In 36 hours local swelling is slightly less, the animal appearing better. Recovery later, with persistence of a slight local swelling.

*Guinea Pig 6.*—0.01 cc. of the same culture as Guinea Pig 4. No evident effects.

*Guinea Pig 7.*—0.1 cc. of a culture from a colony from Guinea Pig 4. Died in 29 hours.

*Autopsy.*—Performed ½ hour post mortem. Usual lesions. Pure cultures from edema, peritoneum, and heart.

*Guinea Pig 8.*—0.02 cc. of the same culture as Guinea Pig 7. Died in 29 hours.

*Autopsy.*—Performed within 1 hour post mortem. The usual lesions. Pure cultures from edema, peritoneum, and heart.

*Rabbit 1.*—2½ cc. of a 2 day chopped meat culture (from culture not passed through animals; rather thin growth). Local swelling in 24 hours. The animal appears somewhat sluggish and ill. Killed with chloroform in 48 hours.

*Autopsy.*—A local abscess in the muscles of the thigh, caused by destruction of muscle tissue, well walled off. The muscles nearby, however, were pale, nearly translucent, crisp, and friable. Further off the muscles were pale and easily torn. Edema was present in the flanks as in the guinea pigs.

*Cultures.*—Pure growth of *B. egens* from the muscle.

*Rabbit 2.*—2 cc. of a culture recently isolated from a guinea pig. In 24 hours slight local swelling. The rabbit appeared sluggish. In 48 hours the local swelling had increased, the leg was apparently paralyzed, and the rabbit appeared extremely sluggish and dull and felt cold to the touch. There was slight crepitation over the local swelling and over the abdomen. Death occurred that night.

*Autopsy.*—At the site of inoculation the muscle was pale externally and on section, friable. The muscles were slightly swollen, and between them were

a dull red pseudogelatinous edema and gas bubbles. A similar edema and gas extended over the abdominal wall and chest. There was marked edema in the flanks and axillæ. The muscles of the chest and abdomen were pale, soft, and friable. *Peritoneal Cavity.*—A small amount of slightly cloudy, pale fluid, containing numerous bacilli.

*Cultures.*—Pure cultures from muscle, edema, peritoneum, and heart.

### *Pathology of the Lesions.*

Sections of muscle from the human case showed muscle degeneration much as in *Bacillus welchii* infection, but without any hemorrhages. There was practically no cellular infiltration. Bacilli were not numerous except in a few areas. No other organisms could be found.

*Kidney.*—Sections showed marked acute tubular degeneration. The nuclei were pyknotic or had disappeared; the protoplasm was swollen and granular. No bacilli were found.

*Liver.*—Fairly well marked degeneration of the central type. No foci of infection.

*Guinea Pig Muscle.*—The infected muscle shows marked edema between muscle fibers, practically no cellular infiltration, and no hemorrhage. The muscle fibers degenerate either in mass, losing structural markings and becoming deep staining, or, and this very frequently, becoming edematous, with separation of the fibrillæ, which still appear sharp and distinct. Bacilli are very numerous between muscle fibers and invade them early.

### *Identity of the Bacillus.*

From other anaerobes than *Bacillus welchii* the bacillus differs morphologically as *Bacillus welchii* does. It is needless to spend much time on this point. It also differs from others in the characters of its colonies. Further differences are given below.

*Bacillus fallax* is motile in culture under favorable conditions and highly motile in animal fluids. *Bacillus egens* is always absolutely immotile. *Bacillus fallax* has scarcely any pathogenic powers and these are quickly lost in subculture. *Bacillus egens* was markedly pathogenic after 6 weeks of subculture. *Fallax* is non-pathogenic for rabbits. When it does produce lesions in guinea pigs they are of a different character. The muscles are red and hyperemic and there

is no muscle lysis. Morphologically *fallax* is thinner, becomes Gram-negative more quickly, has more rounded ends, and does not produce long filaments as described for *Bacillus egens*.

*Bacillus welchii* differs fundamentally by its characteristic stormy fermentation of milk, its vigorous saccharolytic action on maltose, lactose, galactose, saccharose, and glycerol or inulin. Pathologically it does not produce such marked and extensive muscle lysis, and there is often hemorrhage in the lesions, giving a pink or red color to the affected muscles. It is not usually so pathogenic after long subculture, especially for rabbits. Morphologically it is characterized by the absence of filament formation under the conditions described for *Bacillus egens*. Its colonies are quite different.

*Bacillus œdemaciens* differs in morphology (chain formation in cultures and animal tissues; spore formation). In colony characteristics it is absolutely unlike *Bacillus egens*, and the pathological lesions are totally different. There is little or no muscle lysis, but an extensive white jelly-like edema.

*Vibrion septique* differs in motility, morphology, formation of long filaments in peritoneal fluid, spore formation, colony characters, and pathogenic action.

*Bacillus aerofœtidus* is practically non-pathogenic. It is a much smaller, thinner bacillus with much rounded ends, quickly becoming Gram-negative. Its colonies are different and it liquefies blood serum and ferments lactose vigorously.

*Bacillus egens* differs from all the other pathogenic anaerobes in the lack of spore formation and of proteolytic power.

The result of this case shows the importance of careful bacteriological control in testing the use of an anti-gas gangrene serum. It would have been easy to mistake the case for a *Bacillus welchii* infection, especially as *Bacillus welchii* was present on the wound surface, together with other aerobes and anaerobes. Of course the serum could not be expected to have therapeutic value in this particular case.

Whether such an infection as this will prove to be common is uncertain. Its presence would be extremely easy to overlook in the event of a coincident infection with *Bacillus welchii*, for *Bacillus egens* would add scarcely a single positive morphological or cultural character to mixed cultures.

*Nomenclature.*—Since the bacillus appears to be a new species, a name has been given it. Its most evident difference when compared to any other anaerobe consists in the lack of some characteristic; it differs from *Bacillus welchii* by lack of fermentative powers; from *fallax* by lack of motility; from *œdemaciens* and other anaerobes by lack of spore formation and of proteolytic power. Therefore the name *egens* has been chosen.

## BIBLIOGRAPHY.

Weinberg, M., and Séguin, P., La gangrène gazeuse: bactériologie; reproduction expérimentale; sérothérapie, Monograph de l'Institut Pasteur, Paris, 1918.

McIntosh, J., The classification and study of the anaerobic bacteria of war wounds, *Great Britain National Health Insurance, Med. Research Com., Special Rep. Series, No. 12*, 1917, 3.

Henry, H., An investigation of the cultural reactions of certain anaerobes found in wounds, *J. Path. and Bacteriol.*, 1916–17, xxi, 344.

# SPIROCHÆTA HEBDOMADIS, THE CAUSATIVE AGENT OF SEVEN DAY FEVER (NANUKAYAMI).

## Second Paper.

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### INTRODUCTION.

Etiological studies on *nanukayami*, or seven day fever, have been conducted by us in the Prefecture of Fukuoka since the year 1915. In November, 1916, we observed a certain spirochete in a guinea pig which had received an inoculation of blood from a patient having this disease. The spirochete resembled *Spirochæta icterohæmorrhagiæ* in form and motion, but the results of animal experiments and immunological studies undertaken at that time showed it to be a new species and the causative agent of seven day fever. Hence we named it *Spirochæta hebdomadis*.

We have come to know since that this disease occurs not only in the Prefecture of Fukuoka, but also in those of Shizuoka, Okayama, Shiga, Nara, Hyogo, Hiroshima, Kochi, in Kyoto, and elsewhere. These facts were reported in April, 1917, at the meetings of the Nippon Medical Society, the Nippon Pathological Society, and the Nippon Hygienic Society.[1] But the cases of seven day fever which had come under our observation up to that time were few in number, and we were obliged to wait until the next epidemic season of the disease, which was the autumn of that year, in order to continue our investigation.

On September 7, 1917, we went to Sawara County in the Prefecture of Fukuoka. Here by animal experiments made in a number of cases

[1] *Nippon Naika Gakkai Zasshi*, 1917, v, No. 5.

of seven day fever we succeeded in establishing the presence of *Spirochæta hebdomadis* in the blood and in making the discoveries here reported.

A summary of the report made in April, 1917, regarding the causative agent of seven day fever follows:

1. Since 1915, animal experiments had been carried on with six cases of seven day fever. In two cases we used patients' blood taken on the 3rd and 4th days of the disease, in three cases blood from the 6th day, and in one case from the 8th day. The animals employed were rabbits, guinea pigs, white rats, and mice. Three guinea pigs, which had received injections of blood from a patient on the 6th day of the disease, developed fever and died. Spirochetes were found in their livers.

2. The spirochete found resembled *Spirochæta icterohæmorrhagiæ* in form and motion, but the results of animal experiments and immunological studies proved it to be a new species.

3. When a pure culture of this spirochete was inoculated into guinea pigs, they showed symptoms resembling those of seven day fever.

4. In the serum of a patient recovering from seven day fever immune bodies were found which killed and dissolved the spirochete.

5. We proceeded to search for the animal host harboring the organism and discovered the spirochete in the kidneys of wild mice (*Microtus montebelli*). In other words, it was found that wild mice are the carriers of the spirochete, and that they are constantly disseminating it through their urine.

6. There is a close relation between the districts infected with seven day fever and the regions infested with wild mice.

Of all these points, we place the greatest importance upon the immunological observations. The spirochete under discussion, as has been stated, closely resembles *Spirochæta icterohæmorrhagiæ* in form and motility. By means of Pfeiffer's test, however, we found that it was not affected by horse serum immunized with *Spirochæta icterohæmorrhagiæ* or by the serum of a patient convalescing from spirochætosis icterohæmorrhagica. On the other hand, the serum of goats immunized with this spirochete or the serum of a patient recovering from seven day fever failed to act upon *Spirochæta icterohæmorrhagiæ*.

We injected the two immunized sera into guinea pigs infected with either seven day fever or spirochætosis icterohæmorrhagica. The animals inoculated with the homologous immune serum recovered and no spirochetes could be found in their blood, while those

injected with the heterologous immune serum met with the characteristic death of the disease. The spirochetes in the blood were in no way affected by the serum. We inoculated the corresponding spirochetes into guinea pigs which had recovered from seven day fever or spirochætosis icterohæmorrhagica and found that they were not affected, while the animals inoculated with heterologous spirochetes contracted the corresponding disease.

From these observations and on the basis of the modern immunological point of view, we concluded that the spirochete under discussion represents a species different from *Spirochæta icterohæmorrhagiæ*.

We succeeded, therefore, in confirming the causative agent of seven day fever. But in the six cases of the disease which we observed, we were able to confirm the presence of the spirochete in only one. This was probably attributable to the fact that *Spirochæta hebdomadis*, in contrast to *Spirochæta icterohæmorrhagiæ*, does not affect all guinea pigs to the same degree. In heavy animals we found that the inoculation of liver emulsion or pure culture containing even a large number of the spirochetes would not uniformly lead to infection. Only in light weight guinea pigs were we able to produce experimental seven day fever by inoculation. The conclusion was based on the results of the animal experiments referred to, but to test its validity it was necessary that it should be supported by further experiments, with guinea pigs of light weight. As the disease occurs only in the autumn, we had no opportunity to conduct experiments until our visit to Sawara County in 1917.

## EXPERIMENTAL.

The present researches in Sawara County covered the villages of Hara, Kanetake, Takuma, and Iki.[2] The cases of seven day fever which came under our observation from September 7, 1917, to the beginning of November, 1917, numbered twenty-six in all. We proceeded to the infected districts every other day and often made more than one animal experiment on the same patient. Serum was collected when necessary. Of the twenty-six patients under obser-

[2] The patients were studied through the kindness of Dr. Matsuguchi, Dr. Morita, and Dr. Tani of the district.

TABLE I.

*Guinea Pigs Inoculated with the Blood of Seven Day Fever Patients.*

| Case No. | Age. | Sex. | Day of first examination. | Length of time after onset. | No. of guinea pigs used. | Weight of animals. | Results. |
|---|---|---|---|---|---|---|---|
| | *yrs.* | | *1917* | *days* | | *gm.* | |
| 1 | 26 | Male. | Sept. 28 | 1 | 2 | 95 | + |
| | | | | | | 65 | + |
| 2 | 32 | " | Oct. 7 | 2 | 2 | 90 | + |
| | | | | | | 130 | + |
| 3 | 25 | " | Sept. 7 | 3 | 2 | 115 | + |
| | | | | | | 135 | + |
| 4 | 19 | " | " 13 | 3 | 2 | 116 | + |
| | | | | | | 118 | + |
| 5 | 34 | " | " 20 | 3 | 2 | 107 | + |
| | | | | | | 110 | + |
| 6 | 17 | " | " 26 | 3 | 3 | 100 | + |
| | | | | | | 105 | + |
| | | | | | | 105 | + |
| 7 | 20 | " | Oct. 5 | 3 | 2 | 95 | + |
| | | | | | | 110 | + |
| 8 | 31 | " | " 5 | 3 | 1 | 120 | + |
| 9 | 26 | " | " 5 | 3 | 2 | 100 | + |
| | | | | | | 110 | + |
| 10 | 18 | " | " 7 | 3 | 2 | 95 | + |
| | | | | | | 110 | + |
| 11 | 10 | " | Sept. 22 | 4 | 1 | 105 | + |
| 12 | 31 | " | " 26 | 4 | 2 | 100 | + |
| | | | | | | 110 | + |
| 13 | 42 | " | " 28 | 4 | 2 | 85 | + |
| | | | | | | 100 | + |
| 14 | 23 | " | Oct. 5 | 4 | 1 | 100 | + |
| 15 | 18 | Female. | " 9 | 4 | 1 | 140 | − |
| 16 | 16 | Male. | Nov. 5 | 4 | 3 | 90 | + |
| | | | | | | 115 | + |
| | | | | | | 155 | + |
| 17 | 16 | " | Sept. 7 | 5 | 2 | 98 | + |
| | | | | | | 109 | + |
| 18 | 43 | " | " 24 | 5 | 1 | 110 | + |
| 19 | 31 | " | Oct. 5 | 5 | 2 | 95 | + |
| | | | | | | 150 | + |
| 20 | 23 | " | Sept. 11 | 6 | 4 | 85 | + |
| | | | | | | 110 | + |
| | | | | | | 115 | + |
| | | | | | | 118 | − |
| 21 | 17 | " | " 16 | 7 | 4 | 133 | − |
| | | | | | | 142 | − |
| | | | | | | 149 | − |
| | | | | | | 114 | − |
| 22 | 16 | " | Oct. 5 | 7 | 2 | 97 | + |
| | | | | | | 105 | − |
| 23 | 20 | " | " 7 | 7 | 2 | 110 | − |
| | | | | | | 150 | − |

vation, we collected blood from twenty-three. The blood was injected into the peritoneal cavity of guinea pigs ranging in weight from 65 to 150 gm.; most of the animals averaged 100 gm. At least two animals were used for each test, for being small and light they frequently died in the course of the experiment. The quantity of blood injected was about 1 cc. for every 100 gm. of the animal's weight. Table I shows the results obtained from the twenty-three cases.

The twenty-three cases include one of blood taken on the 1st day of the disease, one on the 2nd day, eight on the 3rd day, six on the 4th day, three on the 5th day, one on the 6th day, and three on the 7th day. The guinea pigs inoculated with blood from Case 15, taken on the 4th day, and from Cases 21 and 23 taken on the 7th day, failed to develop fever and the experiments were negative. In the other twenty cases, however, the inoculated guinea pigs all developed fever in 5 to 7 days, and spirochetes were found in the blood. They were isolated in every case, almost in pure culture.

Daily records were kept of the temperatures of the guinea pigs. We also examined by dark-field illumination spirochetes in blood drawn from the ears, to ascertain whether the guinea pigs were affected. The animals were observed closely for symptoms. Only a few of the guinea pigs had icterus, and when it did occur, it was slight, far less severe than in spirochætosis icterohæmorrhagica. The animals were much less apt to bleed. In some of the guinea pigs the spirochetes disappeared from the blood in the course of time; the congestion also disappeared from the orbital conjunctivæ, the temperature fell, and the animals were restored to perfect health. Results such as these have not been observed in animal experiments with *Spirochæta icterohæmorrhagiæ*.

It has been stated already that heavy guinea pigs, those weighing more than 200 gm., are rarely infected with the spirochetes of seven day fever, and even when infected, they seldom die of the disease. This was the finding when one animal was inoculated from another. It remained to be proved, however, whether it was also true of guinea pigs inoculated directly with the blood of patients. We determined to test this point, using guinea pigs of different weights, as shown in Table II, and injecting them with quantities of blood in accordance

with the weight of the animal. The results show that with direct inoculation also the smaller and lighter animals were more easily affected than the heavier ones. Even heavy animals were occasionally affected by the disease, but the percentage was far smaller than with lighter ones. This finding proves that our hypothesis as to the influ-

TABLE II.

*Guinea Pigs of Different Weights Inoculated with the Blood of Seven Day Fever Patients.*

| Experiment No. | Guinea pig No. | Weight. | Amount of blood injected intraperitoneally. | Results. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Spirochetes in blood. | Course. | Autopsy. | Spirochetes in liver. | Spirochetes in kidney. |
| | | *gm.* | *cc.* | | | | | |
| 1 | 1 | 115 | 2.0 | + | Died on 10th day. | + | 5–6 in field. | |
| 2 | 2 | 118 | 2.0 | − | Normal. | | | |
| 3 | 3 | 110 | 2.0 | − | " | | | |
| 4 | 4 | 85 | 2.0 | + | Died on 10th day. | + | 10 in field. | |
| 5 | 5 | 90 | 1.0 | + | Died on 6th day. | + | 10 in field. | |
| 6 | 6 | 95 | 1.0 | − | " " 2nd " | − | − | |
| 7 | 7 | 115 | 1.2 | + | " " 7th " | + | 3–4 in field. | |
| 8 | 8 | 155 | 1.5 | + | " " 8th " | + | 1–2 " " | |
| 9 | 9 | 210 | 2.0 | − | " " 16th " | − | − | − |
| 10 | 10 | 290 | 3.0 | − | " " 15th " | + | − | 1 in 1 specimen. |
| 11 | 11 | 305 | 3.0 | − | " " 4th " | − | − | |
| 12 | 12 | 135 | 1.0 | − | Died on 9th day. | + | 1 in 20–25 fields. | |
| 13 | 13 | 150 | 1.5 | − | " " 9th " | ± | − | |
| 14 | 14 | 185 | 2.0 | − | " " 6th " | − | − | |
| 15 | 15 | 475 | 5.0 | − | " " 10th " | + | 1 in 20–25 fields. | |
| 16 | 16 | 625 | 6.0 | + | " " 29th " | + | − | 1 in 1 specimen. |

ence of weight was in the main correct. When inoculation is carried on in a series from animal to animal, the smaller animals are preferable.

The animal experiments demonstrate that the spirochete under discussion is not the same as *Spirochæta icterohæmorrhagiæ*, but that it agrees in every respect with *Spirochæta hebdomadis*, the discovery of

which we have reported.[3] There is no room left for doubt that the organism obtained from these twenty cases was *Spirochæta hebdomadis*, but for further proof immunological experiments were undertaken. Serum was collected from the twenty patients in the stage of convalescence. This serum, together with the spirochete obtained in 1916 from a patient having seven day fever, and also *Spirochæta icterohæmorrhagiæ*, was subjected to Pfeiffer's test.

Pfeiffer's tests were made in each of the twenty cases. As the results were identical in all, we have reported only five (Tables III and IV). The tests show that the serum of these patients had no effect on *Spirochæta icterohæmorrhagiæ*, while it killed and dissolved *Spirochæta hebdomadis*. It is evident, therefore, that the serum contained immune bodies.

We carried out Pfeiffer's test with the serum from convalescent patients having seven day fever, which contained strong immune bodies both against the spirochetes obtained in 1917 and those of the year previous, and immune serum of *Spirochæta icterohæmorrhagiæ*. As this experiment requires a comparatively large number of guinea pigs, it was confined to ten cases selected from the total number of twenty. We found that the spirochetes of the ten cases were not affected by the immune serum of *Spirochæta icterohæmorrhagiæ*, while the organisms were killed and dissolved by serum obtained from convalescent cases of seven day fever. This serum had already been shown to contain strong immune bodies against the spirochete of 1916.

Pfeiffer's tests were made in ten cases. The results being identical in all, we shall give the records for two only (Tables V and VI).

We believe that the animal experiments and the immunological studies made have proved conclusively that the organisms found in the twenty cases observed in 1917 differ from *Spirochæta icterohæmorrhagiæ*, and that they are of precisely the same species as *Spirochæta hebdomadis* discovered by us the previous year. Hence we are justified in concluding that the causative agent of seven day fever is undoubtedly *Spirochæta hebdomadis* and that the presence of this organism can always be proved by inoculating blood taken

[3] Ido, Y., Ito, H., and Wani, H., *J. Exp. Med.*, 1918, xxviii, 435.

TABLE III.

*Pfeiffer's Tests. Spirochæta hebdomadis Obtained in 1916 and Convalescent Serum from Seven Day Fever.*

| Guinea pig No. | Weight. | Intraperitoneal injection. | | Spirochetes in peritoneal fluid. | | Course. | Results. |
|---|---|---|---|---|---|---|---|
| | | Serum used. | Spirochetes. | After 30 min. | After 2 hrs. | | |
| | *gm.* | | | | | | |
| Control. | 85 | Isotonic salt solution, 1 cc. | *S. hebdomadis*, 1 cc. | 1–2 in field. | 1 in field. | No protection. | − |
| " | 87 | Immune horse, icterohemorrhagic, 1 cc. | *S.* " 1 " | 1–2 " " | 1–2 " " | " " | − |
| " | 143 | Immune, patient's, icterohemorrhagic, 1 cc. | *S.* " 1 " | 1 " " | 1 " " | " " | − |
| " | 104 | Immune, seven day fever (Case 24), 1 cc. | *S.* " 1 " | 0 " specimen. | 0 " specimen. | Protection. | + |
| 17 | 103 | Immune, seven day fever (Case 17), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |
| 18 | 115 | Immune, seven day fever (Case 3), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |
| 19 | 117 | Immune, seven day fever (Case 20), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |
| 20 | 107 | Immune, seven day fever (Case 12), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |
| 21 | 102 | Immune, seven day fever (Case 4), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |

TABLE IV.

*Pfeiffer's Tests. Spirochæta icterohæmorrhagiæ and Convalescent Serum from Seven Day Fever.*

| Guinea pig No. | Weight. | Intraperitoneal injection. | | Spirochetes in peritoneal fluid. | | Course. | Results. |
|---|---|---|---|---|---|---|---|
| | | Serum used. | Spirochetes. | After 30 min. | After 2 hrs. | | |
| | *gm.* | | | | | | |
| Control. | 150 | Isotonic salt solution, 1 cc. | *S. icterohæmorrhagiæ*, 10 in field, 1 cc. | 4–5 in field. | 4–5 in field. | No protection. | − |
| " | 135 | Immune, patient's, icterohemorrhagic, 1 cc. | " " | 0 " specimen. | 0 " specimen. | Protection. | + |
| 22 | 120 | Immune, seven day fever (Case 17), 1 cc. | " " | 4–5 " field. | 2–3 " field. | No protection. | − |
| 23 | 160 | Immune, seven day fever (Case 3), 1 cc. | " " | 3–4 " " | 2–3 " " | " " | − |
| 24 | 170 | Immune, seven day fever (Case 20), 1 cc. | " " | 3–4 " " | 1–2 " " | " " | − |
| 25 | 180 | Immune, seven day fever (Case 12), 1 cc. | " " | 1–2 " " | 2–3 " " | " " | − |
| 26 | 155 | Immune, seven day fever (Case 4), 1 cc. | " " | 1–2 " " | 1–2 " " | " " | − |

TABLE V.

*Pfeiffer's Tests. Spirochetes from Case 4, and Immune Serum of Spirochæta hebdomadis and Spirochæta icterohæmorrhagiæ.*

| Guinea pig No. | Weight. | Intraperitoneal injection. | | Spirochetes in peritoneal fluid. | | Course. | Results. |
|---|---|---|---|---|---|---|---|
| | | Serum used. | Spirochetes. | After 30 min. | After 2 hrs. | | |
| | *gm.* | | | | | | |
| Control. | 95 | Isotonic salt solution, 1 cc. | Spirochetes (Case 4), 10 in field, 1 cc. | 1–2 in field. | 1 in 1–2 fields. | No protection. | − |
| " | 107 | Case 4, 1 cc. | " " | 0 in specimen. | 0 " specimen. | Protection. | + |
| 27 | 110 | Immune, seven day fever (Case 25), 1 cc. | " " | 0 " " | 0 " " | " | + |
| 28 | 110 | Immune, seven day fever (Case 26), 1 cc. | " " | 0 " " | 0 " " | " | + |
| 29 | 88 | Immune, horse, ictero-hemorrhagic, 1 cc. | " " | 1 " 1–2 fields. | 1 " 1–2 fields. | No protection. | − |
| 30 | 100 | Immune, patient's, ictero-hemorrhagic, 1 cc. | " " | 1 " 1–2 " | 1 " 1–2 " | " " | − |

TABLE VI.

*Pfeiffer's Tests. Spirochetes from Case 17, and Immune Serum of Spirochæta hebdomadis and Spirochæta icterohæmorrhagiæ.*

| Guinea pig No. | Weight. | Intraperitoneal injection. | | Spirochetes in peritoneal fluid. | | Course. | Results. |
|---|---|---|---|---|---|---|---|
| | | Serum used. | Spirochetes. | After 30 min. | After 2 hrs. | | |
| | *gm.* | | | | | | |
| Control. | 94 | Isotonic salt solution, 1 cc. | Spirochetes (Case 17), 10 in field, 1 cc. | 3–4 in 1 line.* | 1 in a few fields. | No protection. | − |
| " | 105 | Case 17, 1 cc. | " " | 0 " specimen. | 0 " specimen. | Protection. | + |
| 31 | 103 | Immune, seven day fever (Case 25), 1 cc. | " " | 0 " " | 0 " " | " | + |
| 32 | 120 | Immune, horse, ictero-hemorrhagic, 1 cc. | " " | 1 " 7–8 fields. | 2–3 " 1 line. | No protection. | − |

* Line means from one side to the other of the cover-glass (65 to 70 fields).

from a patient at a proper stage of the disease into guinea pigs of light weight.

In cooperation with Doctors Okuda and Kishimoto we conducted experiments to ascertain the particular day after the onset of the disease when the blood of patients is infective for guinea pigs. Blood was taken from all the patients, usually every other day, for a period of 10 days from the onset. The weight of the animals used for experiment and the method of inoculation were the same as already described. The results are given in Table VII.

TABLE VII.

*Guinea Pigs Inoculated with Blood from 23 Cases of Seven Day Fever.*

| Length of time after onset. | No. of guinea pigs. | Positive. | Negative. | Doubtful. | Per cent positive. | Guinea pigs developing spirochætosis icterohæmorrhagica. | |
|---|---|---|---|---|---|---|---|
| | | | | | | No. of guinea pigs. | Per cent positive. |
| *days* | | | | | | | |
| 1 | 1 | 1 | 0 | 0 | 100 | | |
| 2 | 1 | 1 | 0 | 0 | 100 | 40 | 100 |
| 3 | 8 | 8 | 0 | 0 | 100 | | |
| 4 | 6 | 5 | 1 | 0 | 83.3 | | |
| 5 | 4 | 4 | 0 | 0 | 100 | 7 | 88.9 |
| 6 | 7 | 2 | 4 | 1 | 33.3 | 19 | 73.7 |
| 7 | 12 | 1 | 8 | 3 | 11.1 | 15 | 46.7 |
| 8 | 5 | 0 | 4 | 1 | 0 | 7 | 13.3 |
| 9 | 1 | 0 | 1 | 0 | 0 | 6 | 16.7 |
| 10 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| Totals ... | 46 | | | | | 95 | |

Up to the 3rd day from the onset of the disease ten cases were positive; *i.e.*, 100 per cent of the animals inoculated contracted the disease. On the 4th day some negative results appeared. On the 5th day all four cases were positive; the inoculation experiment showed 100 per cent positive. On the 6th day the percentage of guinea pigs infected with the disease fell considerably, being only 33.3. On the 7th day only 11.1 per cent were positive. On and after the 8th day all were negative, as may be seen in Table VII. The fact that after the 4th day of the disease the experiments on blood inoculation showed negative results may be explained as being attrib-

utable to the appearance in the blood of immune bodies against *Spirochæta hebdomadis*.

An experiment was made to ascertain the time of appearance in the blood of patients of immune bodies which kill *Spirochæta hebdomadis*. Table VIII shows the results.

It will be seen from the table that up to the 5th day Pfeiffer's test failed to prove the appearance of immune bodies. On the 6th and 7th days, however, the test showed the appearance of a large quantity of immune bodies, and on and after the 8th day immunity was complete. Pfeiffer's tests were made, as with *Spirochæta icterohæmorrhagiæ*, with serum obtained from patients and *Spirochæta hebdomadis*. The imperfect immunity referred to in Table VIII

TABLE VIII.

*Time of Appearance of Immune Bodies in the Blood of Seven Day Fever Patients.*

| Length of time after onset. | Positive reactions. | Incomplete reactions. | Negative reactions. | Totals. |
|---|---|---|---|---|
| *days* | | | | |
| 3 | | | 1 | 1 |
| 4 | | | 2 | 2 |
| 5 | | | 2 | 2 |
| 6 | 1 | 4 | 1 | 6 |
| 7 | 7 | 1 | 2 | 10 |
| 8 | 9 | | | 9 |
| 9 | 5 | | | 5 |
| 10 and over. | 2 | | | 2 |

concerns two cases of seven day fever. For Pfeiffer's test in these cases the intraperitoneal fluid collected after 30 minutes and 2 hours respectively was examined by dark-field illumination. In one it was found to differ wholly from control experiments, for after 2 hours no spirochetes could be seen, although the guinea pig was later experimentally infected with the disease. In the other the intraperitoneal fluid also differed from the control. Spirochetes were not completely dissolved and even after 2 hours some of the organisms were still moving slightly.

It is evident that the blood in these cases had begun to lose its infecting power on the 6th day from the onset of the disease. Hence it was to be expected that on and after the 8th day it had become completely negative.

*Spirochæta hebdomadis*, like *Spirochæta icterohæmorrhagiæ*, is found occasionally, although in small numbers only, in film preparations of the blood of patients. We have observed it in four preparations stained by Giemsa's method, one of which was prepared on the 2nd day, two on the 3rd, and one on the 4th day of the disease.

With the cooperation of Doctors Okuda and Kishimoto we ascertained further that the spirochetes of seven day fever are excreted by patients in the urine. A quantity of 40 cc. of urine, taken aseptically from a patient, was centrifugalized and the deposit examined by dark-field illumination. When no spirochetes were observed the deposit was injected into the peritoneal cavity of guinea pigs. Usually after the 8th day of the disease microscopic examination showed the presence of spirochetes in the urine of patients having seven day fever. The only exception was a case where they were found on the 4th day. In the other twenty-one cases examined they were not observed until the 8th day. After the 9th day they were found in comparatively large numbers, and from about the 18th or 19th until the 25th day we found them uniformly. After that day the percentage decreased gradually, but as late as the 39th day we observed active spirochetes. We found a similar condition in spirochætosis icterohæmorrhagica, although in seven day fever the period in which the spirochetes are discharged is somewhat longer. In 40 cases microscopic examination failed to disclose spirochetes. In all these an injection of the deposit was made into the peritoneal cavity, but only two guinea pigs became infected.

Seven day fever is a disease found not only in the Prefecture of Fukuoka, but in other districts of Japan as well. We have established the fact that the "autumnal epidemic" in Shizuoka Prefecture, "*sakushu* fever" in Okayama Prefecture, "autumnal fever" in Kochi Prefecture, a fever found in Kyoto and in the Prefectures of Shiga, Hyogo, Nara, Hiroshima, Miyazaki, Nagasaki, and Formosa are in reality the same disease; *i.e.*, seven day fever. This statement is based on immunological investigations of the serum of patients.[4]

[4] Reported in April, 1917, at the meeting of the Nippon Naika Gakkai.

## CONCLUSIONS.

1. *Spirochæta hebdomadis* is always present in seven day fever and can be confirmed by animal experiments with guinea pigs of light weight.

2. The causative agent of this disease can also be found in film preparations of the blood of patients, though it is not present in large numbers.

3. *Spirochæta hebdomadis* is discharged in the urine of patients having seven day fever. The number of spirochetes in the urine is great during convalescence.

4. Seven day fever is a disease found not only in the Prefecture of Fukuoka, but in many other districts of Japan as well.

We take pleasure in expressing our indebtedness to Professor Inada, under whose direction the studies were undertaken. We also desire to thank our colleagues, Doctors Okuda and Kishimoto, for their assistance.

The studies here reported were carried on in the Laboratory for the Serum Therapy of Spirochætosis Icterohæmorrhagica in connection with Dr. Inada's clinic, established by the Association Commemorating the Tercentenary Anniversary of Toshogu. The experimental animals used were supplied by the Association. We extend our thanks to Prince Iesato Tokugawa, the President of the Association.

# THE USE OF THE FINAL HYDROGEN ION CONCENTRATION IN DIFFERENTIATION OF STREPTOCOCCUS HÆMOLYTICUS OF HUMAN AND BOVINE TYPES.

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*Streptococcus hæmolyticus* is known to cause certain pathologic conditions in man, such as erysipelas, puerperal sepsis, cellulitis, septic sore throat, bronchopneumonia, etc. It is frequently associated with other diseases, often as a secondary invader in measles and scarlet fever. A hemolytic streptococcus of similar characteristics is also found as the etiologic agent in many diseases of lower animals, particularly in mastitis of cows. Furthermore, an organism of like character is almost constantly present in milk and dairy products. In milk-borne epidemics of septic sore throat the weight of evidence supports the view that the disease is caused by human types of hemolytic streptococci which are accidently communicated to the udder of the cow, rather than to the streptococci which are the common cause of bovine mastitis, and which are so frequently found in milk when no disease is present. From an epidemiological standpoint the recognition of distinctive human and bovine types of this organism is of considerable interest and practical importance.

The occurrence of a hemolytic streptococcus, resembling to such a degree the organism found in pathologic lesions of man, has made necessary the devising of methods for differentiation of this type of streptococcus from that of human origin.

The bacteriological diagnosis of these types rests upon the summation of a number of differential features, rather than upon the dis-

tinctive value of any one test. Minor morphological and cultural characters, differences in the thermal death-point, and variations in the degree of hemolytic activity and pathogenicity for rabbits serve to indicate that essential biologic differences exist between these two types. In addition, many observers have noted the fact that non-pathogenic hemolytic streptococci produce more acid in carbohydrate media than the pathogenic varieties. These observations have been based upon the titratable acidity recorded in terms of the amount of normal alkali required to neutralize a given amount of culture fluid.

Some work has been done on the final hydrogen ion concentration as a means of differentiation of these organisms. Clark (1), discussing the diagnostic value of the final hydrogen ion concentration in differentiating cultures of *Bacillus coli*, states: "Sufficient work has been done in this laboratory to indicate that the same phenomena . . . . apply to many of the streptococci." Ayers (2), studying 200 cultures of streptococci, found that two final zones of hydrogen ion concentration were reached: pH. 4.6 to 4.8, 5.5 to 6.0. Smillie (3) records five strains tested by this method, but does not lay stress upon the results obtained. Ayers, Johnson, and Davis (4) have published interesting observations on the limiting hydrogen ion concentration of a series of streptococci, and have found that in dextrose-yeast-peptone medium the strains from pathogenic sources have a lower limiting hydrogen ion concentration than that reached by the non-pathogenic forms. They emphasize again the fact that titratable acidity, as a measure of the fermentative activity of an organism, is influenced so much by the nature and buffer content of the culture medium that this method should be supplanted by the determination of the hydrogen ion concentration. Ayers[1] has suggested the use of the limiting hydrogen ion concentration in differentiation of human and bovine types of streptococci and has found that the organisms obtained from milk gave the high limiting hydrogen ion concentration, that is from pH 4.5 to 5.0, and that most of the cultures were hemolytic.

The present work is confined to a study of hemolytic streptococci, and from it there has developed the fact that the hydrogen ion con-

[1] Personal communication.

centration at which the human strains of *Streptococcus hæmolyticus* cease to grow is different from that which limits growth of hemolytic cultures from bovine sources.

## EXPERIMENTAL.

*Source of Cultures.*—This study comprises 169 strains of *Streptococcus hæmolyticus*, of which 124 were of human origin and 45 from bovine sources. Among the latter are included a number of strains recovered directly from mastitis in cows and others isolated from milk. Two strains are included among these from bovine sources, which were isolated by Smith and Brown (5) from udders of cows during Boston epidemics of septic sore throat, and identified by them as of human type.[2] Nineteen strains came from cream cheese of different brands.

The strains of hemolytic streptococci of human origin were in the majority of instances isolated from the throats, sputum, blood, and lung lesions of men suffering from postmeasles bronchopneumonia, or from the throats of those intimately in contact with these patients during an epidemic of pneumonia at one of the camp hospitals. In addition, several strains of *Streptococcus hæmolyticus* from erysipelas, postpartum sepsis, meningitis, and cellulitis have been included.[3]

*Methods of Isolation and Identification.*—All the cultures were isolated and identified according to the standard methods for the isolation and identification of *Streptococcus hæmolyticus* adopted by the Medical Department of the United States Army. Isolation was made from a single colony on plain blood agar, and subsequent identification confirmed by the hemolysin test, the bile reaction, and the fermentation of certain test substances, as inulin, salicin, etc. The original stock cultures were preserved in plain blood agar stabs or plain blood broth.

[2] The authors acknowledge their indebtedness to Dr. J. Howard Brown and Dr. Frederick S. Jones, of the Department of Animal Pathology of The Rockefeller Institute, Princeton, N. J., for many of these cultures.

[3] Dr. Charles Krumwiede, of the Department of Health of the City of New York, has furnished many of these cultures, and the authors wish to express their appreciation of this courtesy.

*Medium.*—The medium used for the determination of the final hydrogen ion concentration was meat infusion broth with the addition of 1 per cent dextrose. This medium was prepared from the infusion of fresh beef and contained 1 per cent peptone, 0.5 per cent salt, and 1 per cent dextrose. The initial reaction varied from pH 7.6 to 7.8. With the exception of the dextrose, the medium was the same as that used in this laboratory for the routine cultivation of pneumococcus, and the method of preparation was identical with that described by Avery, Chickering, Cole, and Dochez (6).

In all the experiments measured quantities of the dextrose broth were inoculated with uniform amounts of an 18 hour plain broth culture of the organism to be tested and incubated at 37°C.

*Determination of Hydrogen Ion Concentration.*

The colorimetric or indicator method of determining the hydrogen ion concentration of bacterial cultures is, because of its simplicity and availability, the method of choice in studies of the fermentative activity of organisms. The principles and application of this method have been so thoroughly set forth by Michaelis (7) and by Clark and Lubs (8), as well as by other authors, that reference will be made only to the most important details. The color of the culture medium and the turbidity produced by the massive growth of organisms, as well as by precipitation of proteins, have been the principal sources of error. This changing or masking of the indicator color may be obviated in two ways. First, it must be remembered that all successful media are buffer solutions; that is, they contain salts that require relatively large changes in acid or base to produce small changes in the reaction. The hydrogen ion concentration of these solutions is not materially changed by the addition of water. It is possible, therefore, to dilute the color of the medium itself without affecting the reaction. Secondly, the color of the medium may be superimposed upon that of the indicator by the comparator method introduced by Walpole (9) and adopted by Clark and Lubs. The intense turbidity of cultures of *Streptococcus hæmolyticus* grown in dextrose broth may be reduced by use of the supernatant culture fluid, although the combination of the dilution and comparator methods is usually sufficient to insure an accuracy of pH ± 0.1.

The hydrogen ion concentration determinations were made as follows: Duplicate tubes containing 5 cc. each of the culture were diluted with redistilled water to 15 cc. One drop of a 0.1 per cent alcoholic solution of methyl red was then added to one tube while the other tube was used for the comparator color. The hydrogen ion concentration was then read in the comparator block, as modified by Dernby and Avery (10), and recorded to the nearest tenth of a pH. With this indicator the cultures with a reaction more acid than a pH of 4.5 showed a definite claret-red color, while those less acid than a pH of 5.0 showed only a slight salmon tint. This difference is striking. It is important to use the indicator in sufficiently low concentration. The final reaction of the more acid group of organisms is about the lower limit of usefulness of methyl red, that is pH of 4.3, and too much indicator makes it impossible to distinguish between 4.6 and 4.3. Our experience shows that one small drop, about 0.02 cc., gave the sharpest reading. If, however, four or five times as much methyl red is used, the difference in color tints within this narrow zone is much less distinctive. This point cannot be overemphasized, especially since there is a tendency for most workers to use an excess of indicator. The accuracy of the determinations in the range pH 4.6 to 4.0 was controlled in several experiments by the use of the indicator brom phenol blue (tetrabromophenolsulfonephthalein). The standard solutions of known hydrogen ion concentration were prepared for the range 5.8 to 8.0 from phosphates by Sörensen's technique, and for the range 4.0 to 5.8 from acetate by Walpole's directions. The phosphates had been previously standardized by the hydrogen electrode.

*Influence of Dextrose.*

Since Clark and Lubs (11), in using this method for differentiating the types of *Bacillus coli*, have shown that the amount of dextrose available for fermentation by the organism influenced the final reaction, and that if insufficient dextrose was used a reversion of reaction followed, it was necessary to determine the effect of varying concentrations of dextrose upon the final hydrogen ion concentration of *Streptococcus hæmolyticus*. Further, it was desired to determine whether or not the sugar present in the medium has any effect upon the reaction. The following experiment was therefore devised.

To plain broth of known hydrogen ion concentration, dextrose was added in quantities sufficient to make 0.5, 1, and 1.5 per cent dextrose concentration. The medium was incubated over night at 37°C. to test sterility and then inoculated with 0.1 cc. each of an 18 hour plain broth culture of *Streptococcus hæmolyticus*. Broth which had been fermented to remove muscle sugar was used in the same manner.[4] Samples containing 0.5, 1, and 1.5 per cent dextrose were

TABLE I.

*Influence of the Concetrration of Dextrose on the Final Hydrogen Ion Concentration.*

100 cc. bottles inoculated with 0.1 cc. each of 18 hour plain broth cultures of Human 24, Bovine C57, and Cheese 4.

| Series No. | Culture medium. | Initial hydrogen ion concentration. | Hydrogen ion concentration. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 24 hrs. | | | 14 days. | | |
| | | | Human. | Bovine. | Cheese. | Human. | Bovine. | Cheese. |
| | | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| 1 | Plain broth. | 7.6 | 7.35 | 7.25 | 7.15 | 7.3 | 7.1 | 7.3 |
| 2 | " " + 0.5% dextrose. | 7.5 | 5.0 | 4.5 | 4.5 | 5.1 | 4.5 | 4.5 |
| 3 | " " + 1% " | 7.4 | 5.1 | 4.5 | 4.3 | 5.1 | 4.5 | 4.3 |
| 4 | " " + 1.5% " | 7.5 | 5.0 | 4.5 | 4.3 | 5.1 | 4.5 | 4.3 |
| 5 | Sugar-free broth + 0.5% dextrose. | 7.5 | 5.1 | 4.5 | 4.3 | | | |
| 6 | " " + 1% " | 7.5 | 5.1 | 4.5 | 4.3 | 5.1 | 4.5 | 4.3 |
| 7 | " " + 1.5% " | 7.5 | 5.0 | 4.5 | 4.2 | 5.1 | 4.5 | 4.2 |

prepared and inoculated in the same way. The hydrogen ion concentration of the media, after the preliminary sterilization and incubation and before inoculation, was controlled, and 5 cc. samples of the supernatant liquid were removed from the bottles 24 hours and 2 weeks after inoculation (Table I). It is evident from these results that the concentration of dextrose from 0.5 to 1.5 per cent gives the same hydrogen ion concentration, and further, that either plain broth or sugar-free broth is equally available for this purpose. We have, therefore, used throughout the series an ordinary 1 per cent dextrose broth.

[4] In the preparation of sugar-free broth the meat infusion, before the addition of peptone, is fermented by *B. coli* for 18 to 24 hours.

*Rate of Acid Production of Streptococcus hæmolyticus in 1 Per Cent Dextrose Broth.*

It was important to determine the rate of acid production and length of time required to reach the final limiting hydrogen ion concentration. Hence the following experiments were carried out.

1 per cent dextrose broth was prepared from sugar-free medium, as in the previous experiment, and 100 cc. samples were inoculated with two human strains, two cheese strains, and one bovine strain. The cultures were incubated at 37°C., and at definite intervals 5 cc. duplicate samples were removed for examination. The results are given in Table II and plotted in Text-fig. 1.

TABLE II.

*Rate of Acid Production.*

Sugar-free broth + 1 per cent dextrose. Initial pH = 7.6.

| Strain No. | Hydrogen ion concentration. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2½ hrs. | 4¾ hrs. | 6¾ hrs. | 9 hrs. | 10 hrs. | 12 hrs. | 26 hrs. | 15 days. |
| | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| Human 24 | 7.55 | 7.5 | 7.3 | 5.9 | 5.3 | 5.15 | 5.1 | |
| " 43 | 7.5 | 7.5 | 7.3 | 5.7 | 5.25 | 5.15 | 5.1 | |
| Cheese 64 | 7.45 | 7.05 | 5.1 | 4.8 | 4.5 | 4.3 | 4.3 | 4.3 |
| " 2 | 7.45 | 7.1 | 5.1 | 4.5 | 4.5 | 4.4 | 4.4 | |
| Bovine C57 | 7.5 | 7.5 | 6.9 | 5.3 | 4.95 | 4.6 | 4.5 | 4.4 |

It is evident from these results that the organisms from all three sources reached their final hydrogen ion concentration within 24 hours. The values for the duplicate cultures were so close that only one curve for each set is apparent. Although the curve for acid production of these organisms had reached the maximum within 24 hours, it seemed desirable to determine whether or not continued incubation produced any change in the hydrogen ion concentration. For this purpose nine strains were inoculated in 100 cc. portions of 1 per cent dextrose broth and incubated at 37°C. (Table III). These results substantiate the preceding experiments in that the final hydrogen ion concentration is reached within 24 hours, and show further that this final hydrogen ion concentration does not change on continued incubation. Although 14 days was the longest period




TEXT-FIG. 1. Rate of acid production of human and bovine types of *Streptococcus hæmolyticus*.

of test in this series, the same results have been obtained from cultures tested after 3 weeks.

TABLE III.

*Effect of Prolonged Incubation.*

100 cc. of sugar-free broth + 1 per cent dextrose inoculated with 0.05 cc. of 18 hour plain broth cultures. Initial pH = 7.3.

| Strain No. | Source. | Hydrogen ion concentration. | | | | |
|---|---|---|---|---|---|---|
| | | 18 hrs. | 2 days. | 4 days. | 7 days. | 14 days. |
| | | *pH* | *pH* | *pH* | *pH* | *pH* |
| 24 | Human. | 5.2 | 5.1 | 5.1 | 5.1 | 5.1 |
| 66 | " | 5.2 | 5.2 | 5.2 | 5.1 | |
| 2 | " | 5.1 | 5.1 | 5.1 | 5.1 | |
| 43 | " | 5.3 | 5.1 | 5.1 | 5.1 | |
| 14 | " | 5.2 | 5.1 | 5.15 | 5.1 | |
| 118 | " | 5.2 | 5.2 | 5.1 | 5.1 | |
| 276 | " | 5.2 | 5.1 | 5.1 | 5.1 | |
| 277 | " | 5.2 | 5.1 | 5.1 | 5.1 | 5.1 |
| 11 | " | 5.15 | 5.1 | 5.1 | 5.1 | 5.1 |

*Effect of Animal Passage upon the Final Hydrogen Ion Concentration.*

Since the question of virulence is always of first importance in a discussion of the biology of an organism, it was deemed advisable to determine whether or not animal passages had any effect upon the final hydrogen ion concentration. To determine this five original cultures and their corresponding subcultures, which had been passed through a number of animals, were selected and inoculated in 100 cc. portions of 1 per cent dextrose broth. The results are given in Table IV. It is evident from this experiment that the final hydrogen ion concentration of pathogenic *Streptococcus hæmolyticus* is not affected by animal passage.

*Constancy of the Final Hydrogen Ion Concentration of the Same Strains in Different Experiments.*

The constancy of the final hydrogen ion concentration of *Streptococcus hæmolyticus* is strikingly shown in Table V in which the hydrogen ion concentration of the same strains, but of different experiments, have been assembled.

TABLE IV.

*Influence of Animal Passage on the Final Hydrogen Ion Concentration.*

| Strain No. | No. of animal passages. | Hydrogen ion concentration. | | |
|---|---|---|---|---|
| | | 24 hrs. | 48 hrs. | 8 days. |
| | | *pH* | *pH* | *pH* |
| 1 | | 5.1 | 5.1 | 5.1 |
| 1 | 7 | 5.3 | 5.3 | 5.3 |
| 23 | | 5.1 | 5.1 | 5.1 |
| 23 | 16 | 5.15 | 5.1 | |
| 276 | | 5.15 | 5.1 | 5.1 |
| 276 | 25 | 5.1 | 5.1 | 5.1 |
| 24 | | 5.3 | 5.15 | 5.1 |
| 24 | 25 | 5.1 | 5.1 | 5.1 |
| 84 | | 5.1 | 5.1 | 5.1 |
| 84 | 16 | 5.3 | 5.3 | 5.3 |

TABLE V.

*Constancy of the Final Hydrogen Ion Concentration of the Same Strains in Different Experiments.*

1 per cent dextrose in all media.

| Experiment No. | Strain No. | | | | | |
|---|---|---|---|---|---|---|
| | Human. | | Bovine. | | Cheese. | |
| | 24 | 8 | C53 | C57 | 4 | 1 |
| | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| 1 | 5.1 | | | | | |
| 2 | | 5.2 | | | | |
| 3 | | | 4.4 | 4.4 | 4.3 | 4.3 |
| 4, *a* | 5.15 | 5.1 | | | | |
| 4, *b* | 5.1 | 5.3 | | | | |
| 5 | | | 4.3 | 4.3 | 4.3 | 4.3 |
| 6 | 5.1 | | | 4.5 | 4.3 | 4.3 |
| 7 | | | 4.5 | 4.5 | | |
| 8 | 5.1 | | | 4.5 | 4.3 | |
| 9 | 5.1 | | | 4.5 | 4.3 | |
| 10 | | | | | 4.5 | |

TABLE VI.

*Final Hydrogen Ion Concentration of Streptococcus hæmolyticus of Known Human Origin.*

| Strain No. | Source. | Diagnosis. | pH |
|---|---|---|---|
| 1 | Autopsy (lung). | Bronchopneumonia following measles. | 5.2 |
| 5 | " " | " " " | 5.1 |
| 20 | " " | " " " | 5.1 |
| 29 | Sputum. | " " " | 5.15 |
| 32 | Autopsy (lung). | " " " | 5.0 |
| 39 | " " | " " " | 5.2 |
| 59 | " (blood). | " " " | 5.1 |
| 78 | | " " " | 5.2 |
| 92 | Pleural fluid. | " " " | 5.2 |
| 93 | | " " " | 5.1 |
| 97 | Pleural fluid. | " " " | 5.1 |
| 107 | Sputum. | " " " | 5.3 |
| 110 | | " " " | 5.1 |
| 113 | | " " " | 5.1 |
| 118 | Pleural fluid. | " " " | 5.2 |
| 136 | | " " " | 5.0 |
| 137 | Throat. | " " " | 5.1 |
| 138 | Sputum. | " " " | 5.1 |
| 142 | | " " " | 5.1 |
| 145 | Throat. | " " " | 5.1 |
| 149 | Blood. | " " " | 5.1 |
| 151 | Pleural fluid. | " " " | 5.1 |
| 49 | Sputum. | " " German measles. | 5.1 |
| 2 | Autopsy (lung). | " | 5.1 |
| 3 | " " | " | 5.1 |
| 14 | " " | " | 5.1 |
| 24 | " " | " | 5.1 |
| 50 | Pleural fluid. | " | 5.1 |
| 55 | " " | " | 5.0 |
| 67 | Blood. | " | 5.1 |
| 72 | Throat. | " | 5.1 |
| 84 | Pleural fluid. | " | 5.2 |
| 139 | Throat. | " | 5.1 |
| 143 | Pleural fluid. | " | 5.2 |
| 152 | " " | " | 5.1 |

TABLE VI—*Continued.*

| Strain No. | Source. | Diagnosis. | pH |
|---|---|---|---|
| 10 | Throat. | Measles. | 5.1 |
| 37 | " | " | 5.0 |
| 43 | " | " | 5.1 |
| 47 | " | " | 5.1 |
| 53 | " | " | 5.1 |
| 60 | " | " | 5.1 |
| 64 | " | " | 5.1 |
| 66 | " | " | 5.2 |
| 71 | " | " | 5.1 |
| 79 | " | " | 5.1 |
| 82 | " | " | 5.0 |
| 83 | " | " | 5.1 |
| 85 | " | " | 5.1 |
| 86 | " | " | 5.1 |
| 88 | " | " | 5.1 |
| 89 | " | " | 5.1 |
| 96 | " | " | 5.2 |
| 98 | " | " | 5.2 |
| 108 | " | " | 5.1 |
| 116 | " | " | 5.2 |
| 117 | " | " | 5.1 |
| 119 | " | " | 5.0 |
| 126 | " | " | 5.2 |
| 127 | " | " | 5.1 |
| 128 | " | " | 5.1 |
| 141 | " | " | 5.1 |
| 148 | " | " | 5.15 |
| 150 | " | " | 5.1 |
| 4 | " | German measles. | 5.0 |
| 28 | " | " " | 5.1 |
| 38 | " | " " | 5.0 |
| 45 | " | " " | 5.1 |
| 47 | " | " " | 5.1 |
| 48 | " | " " | 5.1 |
| 54 | Sputum. | " " | 5.0 |
| 70 | Throat. | " " | 5.1 |
| 115 | " | " " | 5.1 |
| 134 | " | " " | 5.1 |
| 140 | " | " " | 5.1 |

TABLE VI—*Continued.*

| Strain No. | Source. | Diagnosis | pH |
|---|---|---|---|
| 9 | | Lobar pneumonia. | 5.1 |
| 11 | Sputum. | " " (Type I). | 5.1 |
| 15 | Throat. | " " | 5.1 |
| 16 | Sputum. | " " | 5.2 |
| 27 | Autopsy (lung). | " " | 5.1 |
| 44 | Throat. | " " | 5.1 |
| 56 | Autopsy (lung). | " " | 5.1 |
| 61 | Sputum. | " " (Type IV). | 5.2 |
| 62 | Throat. | " " | 5.1 |
| 65 | Sputum. | " " | 5.1 |
| 69 | Autopsy (lung). | " " | 5.0 |
| 75 | | " " | 5.1 |
| 87 | Sputum. | " " | 5.2 |
| 95 | Throat. | " " | 5.1 |
| 99 | | " " | 5.1 |
| 120 | Throat. | " " | 5.1 |
| 125 | " | " " | 5.1 |
| 133 | | " " | 5.1 |
| 144 | Throat. | " " | 5.1 |
| 8 | " | Pneumonia. | 5.1 |
| 281 | Sputum. | " | 5.1 |
| 286 | Pleural fluid. | " | 5.1 |
| 6 | Throat. | Incipient tuberculosis. | 5.1 |
| 34 | " | " " | 5.1 |
| 41 | " | " " | 5.1 |
| 46 | " | " " | 5.1 |
| 121 | " | " " | 5.2 |
| 122 | " | " " | 5.1 |
| 129 | " | " " | 5.1 |
| 40 | Pericardial fluid. | | 5.0 |
| 267 | Foot. | Cellulitis. | 5.0 |
| 271 | Blood. | Septicemia. | 5.0 |
| 276 | Pus. | Pelvic abscess. | 5.1 |
| 277 | " (abdomen). | | 5.1 |
| 264 | Blood. | Osteomyelitis. | 5.2 |
| 266 | " | Meningitis. | 5.0 |
| 306 | Spinal fluid. | " | 5.0 |

TABLE VI—*Concluded.*

| Strain No. | Source. | Diagnosis. | pH |
|---|---|---|---|
| SH | 1913 Boston epidemic. | Peritonitis. | 5.0 |
| S8 | 1917 " " | Empyema. | 5.2 |
| 38 | 1912 Baltimore " | Adenitis. | 5.0 |
| C64B | Throat. | | 5.1 |
| C63B | " | | 5.1 |
| 57* | " | Normal. | 4.8 |
| 91 | " | Measles. | 4.8 |
| 103 | " | " | 4.9 |
| 105 | " | Influenza. | 4.9 |
| 288 | Sputum. | Pneumonia. | 4.8 |
| X35B | | Erysipelas. | 4.9 |
| A103B | Throat. | | 4.8 |
| 30 | 1912 Chicago epidemic. | Peritonitis. | 4.9 |

* The eight human strains with a pH of 4.9 and 4.8 have been grouped together without reference to diagnosis.

TABLE VII.

*Final Hydrogen Ion Concentration of Streptococcus hæmolyticus from Cheese.*

| Strain No. | pH | Strain No. | pH |
|---|---|---|---|
| Ch. 1 | 4.3 | Ch. 11 | 4.4 |
| " 2 | 4.4 | " 12 | 4.5 |
| " 3 | 4.3 | " 13 | 4.4 |
| " 4 | 4.4 | " 14 | 4.4 |
| " 5 | 4.4 | " 15 | 4.5 |
| " 6 | 4.3 | " 16 | 4.3 |
| " 7 | 4.5 | " 17 | 4.5 |
| " 8 | 4.3 | " 18 | 4.3 |
| " 9 | 4.5 | " 19 | 4.5 |
| " 10 | 4.5 | | |

## *Survey of Streptococcus hæmolyticus.*

With the assurance given that the media and conditions of growth were suitable for a survey of streptococci from various sources, we have completed the examination of 124 strains of *Streptococcus hæmolyticus* of known human origin and 45 from bovine sources, including 19 cultures of hemolytic streptococci isolated from cheese and 26 from milk. These results have been assembled in Tables VI to VIII, and are represented graphically in Text-fig. 2.

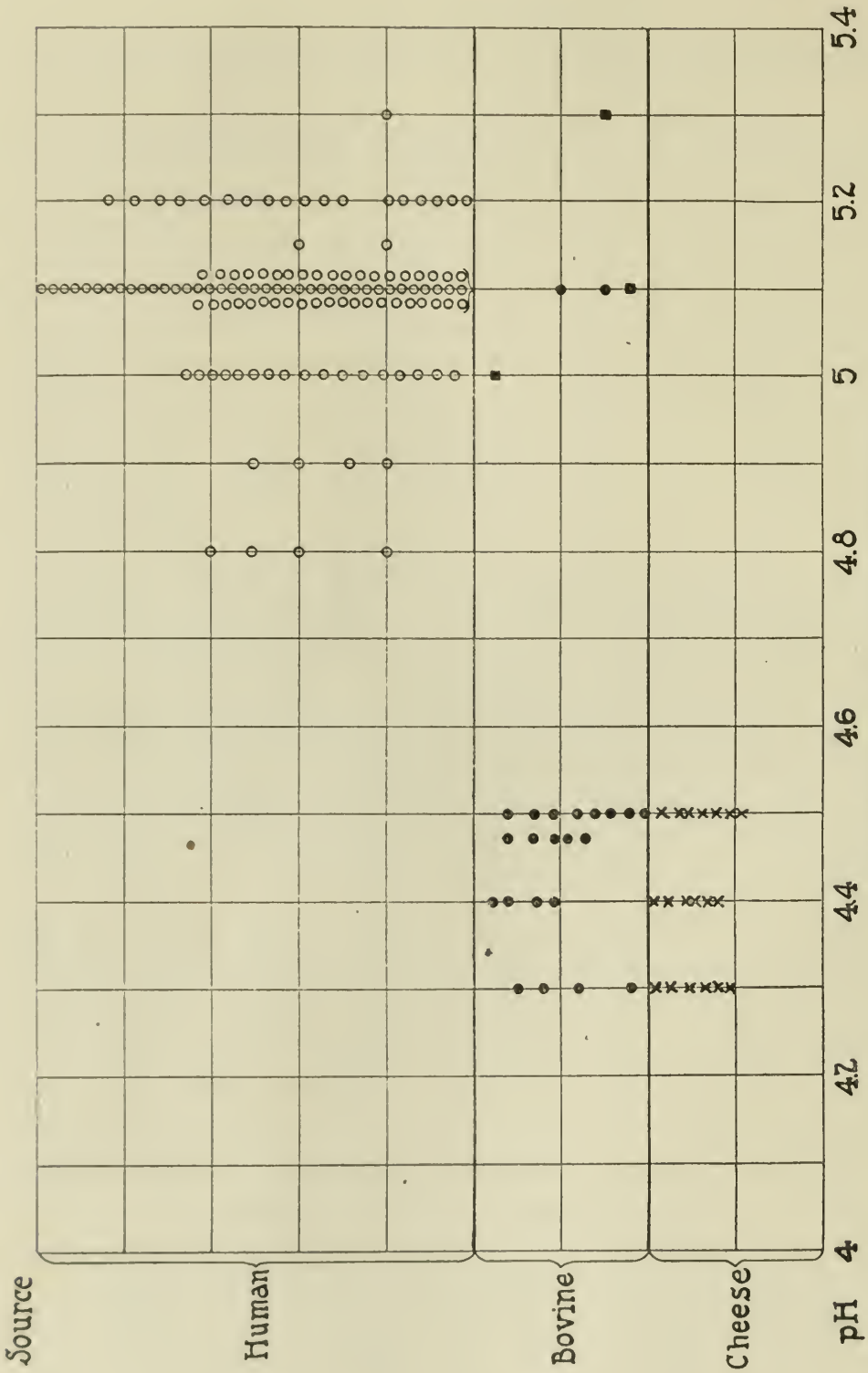
TABLE VIII.

*Final Hydrogen Ion Concentration of Streptococcus hæmolyticus from Bovine Sources.*

| Strain No. | Source. | pH | Strain No. | Source. | pH |
|---|---|---|---|---|---|
| C67 | Mastitis. | 4.5 | M2 | Milk. | 4.3 |
| C57 | " | 4.5 | M41 | " | 4.5 |
| C53 | " | 4.5 | M43 | " | 4.5 |
| C69 | " | 4.5 | V1 | Udder. | 4.4 |
| C59 | " | 4.3 | V2 | " | 4.3 |
| M26 | " | 4.5 | V3* | " | 5.0 |
| M93 | Milk. | 4.5 | V4 | " | 4.3 |
| M53* | " | 5.3 | V5 | " | 4.5 |
| M3 | " | 4.5 | V6 | Milk. | 4.5 |
| M86* | " | 5.1 | V7 | Udder. | 4.4 |
| MJ1* | " | 5.1 | V8 | " | 4.5 |
| M65 | " | 4.5 | V9 | " | 4.5 |
| M1 | " | 4.4 | V10* | " | 5.1 |

* Strains V3 and V10 were isolated by Smith and Brown (5) from the udders of cows during Boston epidemics of septic sore throat and identified by them as human types. Strains MJ1, M86, and M53 were recovered by Jones from milk of cows showing no evidence of mastitis, and were described by him as either human strains which had contaminated the milk or streptococci from the skin or feces of cows. None of these cultures has been tested for pathogenicity. He found that none of the three strains agglutinated in the immune serum prepared by immunization of animals with *Streptococcus hæmolyticus* from bovine mastitis (12).

The results from this study are striking. None of the cheese strains shows a final hydrogen ion concentration less than a pH of 4.5. Of the 26 cultures from bovine sources, 21 had a final reaction between 4.3 and 4.5. Five showed a reaction of from 5.0 to 5.3; of these 5, 2 were received from Dr. J. H. Brown, and were designated by him, from their cultural characteristics, as human streptococci. The other 3 are of questionable diagnosis (foot-note, Table VIII). Of the 124 strains from known human origin, only 8 showed a pH less than 5.0 and none less than 4.8. It must be emphasized that the final hydrogen ion concentration of none of the large number of organisms examined occurred within the zone of 4.5 to 4.8, and only 8 in the zone of 4.8 to 5.0. This is important, for the zone below a pH of 4.5 showed a distinct red color with methyl red, while a reaction above 5.0 showed only a very faint salmon pink.




TEXT-FIG. 2. Difference in final hydrogen ion concentration of human and bovine types of *Streptococcus hæmolyticus*.

*Practical Application of the Method of Hydrogen Ion Concentration in the Differentiation of Human and Bovine Types of Streptococcus hæmolyticus.*

The difference in the final hydrogen ion concentration of these two types of organisms has been expressed in terms of the pH values throughout the present discussion. In the practical application of this method, however, it is not necessary to determine the actual final hydrogen ion concentration. Methyl red reacts in such a way at the different final hydrogen ion concentrations reached by human and bovine strains, that no difficulty is experienced in judging these color values directly. At a pH value of 5.0 to 5.2, representing the range of the final hydrogen ion concentration of human strains, the color of this indicator is a faint salmon pink, whereas at a pH of 4.3 to 4.5, the final value for organisms of bovine origin, the color is a decided red. This color difference is so marked that comparison with standard solutions is not necessary and slight experience suffices to permit the determination of the type from the color produced by the direct addition of methyl red to the culture.

In determining the type *Streptococcus hæmolyticus* the following procedure has been adopted. The strain to be tested is grown in test-tubes (7 by $\frac{7}{8}$ inches) containing 5 cc. of 1 per cent dextrose broth. After the maximum growth has been reached, generally within 24 to 48 hours depending upon the size of the inoculum and the suitability of the medium, the culture fluid is diluted with 10 cc. of distilled water, and one drop of 0.1 per cent alcoholic solution of methyl red added. The difference in color, representing the pH values, distinctive for the human and bovine types of *Streptococcus hæmolyticus* is immediately apparent. This reaction is sufficiently constant to be relied upon, and although an occasional atypical strain may be encountered, it constitutes a presumptive test of real value in differential diagnosis.

## DISCUSSION.

The bacteriological methods used at present in the differentiation of human and bovine types of *Streptococcus hæmolyticus*, namely the

determination of their hemolytic and fermentative activity, and pathogenicity for rabbits, are time-consuming and inadequate. In addition, the determination of the titratable acidity produced by these organisms in the fermentation of certain test substances is admittedly unreliable. The corelation of these several reactions, however, together with minor morphological and cultural characters indicates clearly that distinctive differences of a biologic nature exist between hemolytic streptococci found in pathologic lesions of man and those occurring in bovine mastitis, in milk and dairy products. Any method, therefore, for the rapid differentiation of these closely allied organisms would be of inestimable advantage in epidemiological investigation.

From the data recorded in these experiments it appears that the final hydrogen ion concentration of human and bovine hemolytic streptococci is a constant and distinctive characteristic of these organisms. The human type of *Streptococcus hæmolyticus* reaches a final hydrogen ion concentration of pH 5.2 to 5.0, and the bovine type of pH 4.5 to 4.3. It has been found that in the medium described, the concentration of dextrose, 0.5 to 1.5 per cent, does not affect the final hydrogen ion concentration. Acid production in dextrose medium proceeds rapidly and the maximum acidity is reached in 24 to 48 hours, depending on the size of the inoculum and suitability of the medium for growth. This final reaction inhibits growth and does not change on further incubation of the culture. It also appears from these experiments that the final hydrogen ion concentration of *Streptococcus hæmolyticus* is not related to virulence, since no variation was found in the reaction of cultures as originally isolated and of the same cultures after repeated animal passage.

All the cultures studied were of known origin, and on the basis of clinical evidence and bacteriological methods had been previously identified as belonging to one or the other of these two types. By the method described 95 per cent of all the strains examined were accurately and rapidly classified by the determination of their final hydrogen ion concentration in 1 per cent dextrose broth.

The final hydrogen ion concentration of the strains from bovine sources has in no instance fallen in the intermediary zone between the limits distinctive for human and bovine types. Of the five strains of bovine origin giving the same final hydrogen ion concentration

as human strains, two had previously been identified by other methods as belonging to the human type. The other three may be either human strains which have contaminated the milk or streptococci from the skin or feces of cows (foot-note, Table VIII). Further investigation should be made on the final hydrogen ion concentration of hemolytic streptococci from feces and skin of cows.

In the practical application of these facts use has been made of the difference in color of methyl red at the final hydrogen ion concentration of these two types. This change in color is sufficiently distinctive in itself to serve as a direct test for type determination and obviates the necessity of comparative readings with accurately standardized solutions of the phosphates. Because of the accuracy and constancy of the final hydrogen ion concentration, this method offers a presumptive test of distinct value in the classification of hemolytic streptococci.

## SUMMARY.

1. Under the conditions of these experiments, there appears to be a distinct and constant difference in the final hydrogen ion concentration of *Streptococcus hæmolyticus* from human and bovine sources.

2. Of 124 strains of *Streptococcus hæmolyticus* from known human origin, 116 reached a final hydrogen ion concentration of from pH 5.0 to 5.3. Only 8 reached a pH more acid than 5.0 and none more acid than pH 4.8.

3. Of 45 strains of *Streptococcus hæmolyticus* from bovine sources, including 26 strains isolated from milk and the udder of cows, and 19 from cream cheese, 40 reached a final hydrogen ion concentration of pH 4.3 to 4.5. Of the remaining 5 which reached a pH of 5.0 to 5.2, two were of known human type and three of uncertain diagnosis.

4. A rapid and practical application of this method is proposed as a presumptive test in the differentiation of human and bovine types of *Streptococcus hæmolyticus*.

## BIBLIOGRAPHY.

1. Clark, W. M., *J. Biol. Chem.*, 1915, xxii, 87.
2. Ayers, S. H., *J. Bacteriol.*, 1916, i, 84.
3. Smillie, W. G., *J. Infect. Dis.*, 1917, xx, 45.
4. Ayers, S. H., Johnson, W. T., Jr., and Davis, B. J., *J. Infect. Dis.*, 1918, xxiii, 290.
5. Smith, T., and Brown, J. H., *J. Med. Research*, 1914–15, xxxi, 455.
6. Avery, O. T., Chickering, H. T., Cole, R., and Dochez, A. R., Acute lobar pneumonia. Prevention and serum treatment, Monograph of The Rockefeller Institute for Medical Research, No. 7, New York, 1917.
7. Michaelis, L., Die Wasserstoffionenkonzentration, Berlin, 1914.
8. Clark, W. M., and Lubs, H. A., *J. Bacteriol.*, 1917, ii, 1.
9. Walpole, G. S., *Biochem. J.*, 1911, v, 207.
10. Dernby, K. G., and Avery, O. T., *J. Exp. Med.*, 1918, xxviii, 345.
11. Clark, W. M., and Lubs, H. A., *J. Infect. Dis.*, 1915, xvii, 160; *J. Biol. Chem.*, 1917, xxx, 209.
12. Jones, F. S., *J. Exp. Med.*, 1918, xxviii, 253.

# THE TEMPERATURE OF ACUTELY INFLAMED PERIPHERAL TISSUE.

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## INTRODUCTION.

The rise in the local temperature observed in peripheral areas of the body at the site of acute inflammation is generally attributed to the increased afflux of blood in these parts. This assertion is supported by the finding that in general the temperature of the inflamed peripheral tissue is not greater than the temperature of the internal parts and by the clinical observation that the temperature of the inflamed center is in proportion to the degree of the inflammatory hyperemia present. The fact is therefore excluded, and has recently been denied (1) that the inflamed center should have in reality a temperature higher than that of a corresponding area. The heat of the part has been attributed to the hyperemia.

The argument is of considerable theoretic importance and was discussed during the whole of the past century. The older treatises devoted many pages to the point, which appears now to have been solved as has already been stated. The subject has, however, scarcely received mention in the text-books, which as a rule paraphrase the statement of Cohnheim (2) made in his manual.

## HISTORICAL.

The last known experiments on this point date from the year 1885. At that time Maximow demonstrated by a series of accurate determinations carried out with thermoelectric batteries that in no part of the inflammatory process is the temperature higher than that of the heart's blood or the blood of the larger vessels. Maximow thus confirmed what had been proved previously by Jacobson (3), Bernard (4), Schneider (5), and Huppert (6); namely, that if the inflamed center has a temperature higher than that of the corresponding area, it cannot be asserted that its temperature is above that of the central blood.

The older literature has a treatise by Weber which appealed to Liebermeister (7) who was conservative on this point, which Cohnheim (8) overlooked. Cohnheim merely stated that others more competent than himself had contradicted his statements.

Weber stated his experimental findings as follows: Measuring with thermoelectric batteries the temperature of the vein and that of the artery of the inflamed area, it is found that the venous blood is warmer than either the arterial blood from the same part or the venous blood from corresponding parts not inflamed.

The oblivion into which the controversy has fallen, particularly Weber's treatise, is demonstrated by the report of Galeotti (9), wherein Hunter, Weber, and Simon are mentioned as being of the same opinion, while in fact Hunter opposed the ideas of Weber and Simon.

## *The Problem.*

I have failed to find that Weber's experiment was technically in error. It would seem to be a simple matter to establish whether the blood by its passage through an inflamed part gives off or absorbs heat. On the other hand, an impartial analysis of the problem leaves us uncertain as to the demonstrative value of the experiments mentioned in solving the point.

Considering the mass of tissue in the peripheral area in comparison with the volume of blood which circulates there, and bearing in mind also that the specific heat of blood is much like that of water, one is inclined to ask whether the blood is not a disturbing element, inasmuch as it conceals the real temperature of the inflamed center, giving to the part its own temperature in proportion to the blood volume which is renewed continually.

With regard to the liver, for instance, which has a much larger mass than a small inflamed area has, Claude Bernard proved that the circulating blood carries off heat from the parenchyma. Cavazzani measured an increase of over half a degree in the parenchyma when the hepatic artery and the portal vein were blocked. In cases of parotitis the classic experiments of Ludwig and Spiess (10) confirmed by Bayliss and Hell and Burton-Opitz (11) demonstrate an almost analogous fact: marked local heating and a temperature elevated above that of the arterial blood, with the venous blood warmer than the arterial blood.

It appears, therefore, that the experiment which I call Maximow's, although others have done it before him, cannot be considered exhaustive. On the other hand, our increased knowledge regarding the histology of the inflammatory process—and we know that there is an intense local metabolism—favors the idea that such metabolism may be accompanied by an increased release of heat, especially in view of the fact that most of the biologic reactions, as we know them, are strikingly disintegrating and isothermal in their effects. Virchow shared this view when he asserted the histogenetic origin and nature of the inflammatory process. Courmont (12) stated that to exclude all local production of heat contradicted accepted ideas.

Among the numerous authors who believe that the temperature of the inflamed area depends upon the increased afflux of blood, none has reflected on the physiological postulate that in general the tissues warm the blood and that it is not the blood which gives heat to the tissues. Claude Bernard (13) in discussing this point presented the problem as to whether the flow of blood to a part might not be considered the result of local calorification.

## EXPERIMENTAL.

It seemed inadvisable to repeat Weber's experiment, because of the difficulties encountered in carrying it out accurately. It is necessary to be constantly near the animal under experimentation and also to make manipulations in the immediate vicinity of the thermoelectric batteries, which may readily lead to errors. Anyone who has had experience with this delicate instrument will acknowledge the difficulty of avoiding radiations of heat. Moreover, according to Weber's technique, it is necessary to introduce one electrode into the lumen of an artery and the other into that of an important vein. The possibility is always present that by this method the afflux and efflux of blood in the part may be altered in an indeterminable, irregular manner.

In a problem of this nature, with opinions so greatly at variance and so strongly impressed in the minds of pathologists, the services of expert technicians are required in order to eliminate criticism. In my experiments the difficulty has in the main been surmounted by regarding the blood not as the criterion, but rather as a disturbing element.

In the experiments presented here the attempt was made to compare the temperature of the inflamed tissue with that of the corresponding healthy area, at the moment when the circulation of blood was definitely arrested by the death of the animal or temporarily by ischemia. The temporary arrest of the circulation for thermostatic purposes is not a new device. Kussmaul and Tenner (14) tried it as far back as 1857 in the neuroparalytic hyperemia produced by incision of the sympathetic nerve in the area not inflamed. Cavazzani, as already stated, more recently used the same measure to establish the thermogenetic power of the liver.

The experience of other observers served as a guide in the choice of an indicator. Thermoelectric batteries, if connected to a sensitive measuring apparatus, are preferable to mercury thermometers which are slow, bulky, and require direct reading.[1]

### *Technique.*

The electromotor force developed on the contact surface between two metals placed at different temperatures is indicated below. The electromotor force developed by the contact of two metals is equal to the difference between the values registered against them. For instance, the electromotor force developed between patent nickel and constantan is 8, between bismuth and antimony 100.

*Electromotor Force Developed between Two Metals.*

| | |
|---|---|
| Bismuth | 0 |
| Constantan | 30 |
| Patent nickel | 38 |
| Nickel | 51 |
| Platinum rhodium | 61 |
| Platinum iridium | 61 |
| Platinum | 66 |
| Mercury | 67 |
| Gold, silver | 74 |
| Iron | 83 |
| Antimony | 100 |

[1] I am indebted to Professor V. Grandis, Director of the Department of Physiology of the University of Genoa, for his courtesy in teaching me the technique and for his supervision of some of the experiments.

As in our experiments we were obliged to use thin wires, we could not employ bismuth-antimony electrodes, and on the other hand, not finding a sufficient quantity of constantan of the required diameter, Professor Grandis prepared from a disc of patent nickel (German nickeline) a long, thin wire which he soldered to a soft, iron wire as shown in the following diagram:




*A* and *B* were flattened and reduced to a lanceolated form. The highest possible potential was 45 microvolts for each degree of difference between 0 and 100. For measuring the developed potential we employed a Thompson galvanometer, to which the battery was attached directly after the internal resistance in respect to the inserted system had been regulated. After a number of attempts we succeeded in getting exact readings; *i.e.*, to 0.1 of a degree. This was done by adjusting the scale of the galvanometer. By means of this sensitive scale, the deviations did not require correction on direct reading, because the variations of temperature in the limits of observation were proportional to the angle of deviation of the galvanometer. This finding is corroborated by a number of observers.

To avoid abnormal radiations of heat which might lead to errors, the animal under experimentation was kept in a large, isolated room especially designed for calorimetric researches; here the temperature was practically constant. The readings of the valuations of potentiality were made in an adjoining room.

An inflammatory lesion was created by breaking, under ether anesthesia, the femur of a guinea pig without, however, injuring the skin. Inflamed areas appeared not earlier than 4 days later, or later than 12 days.

For the experiment one of the electrodes was introduced into the inflamed area of the guinea pig, while the other was inserted in a corresponding area on the other side. In general the introduction of the electrodes was not followed by considerable hemorrhage; when, however, hemorrhage did occur, the experiment was discontinued.

The system having been properly installed, the guinea pig was killed quickly by puncturing the medulla, or temporary ischemia of

the posterior part of the aorta was produced by compressing the thoracic portion. In the latter case, every precaution was taken to see that the heat from the compressing hand did not radiate to the electrodes. A bolometer was also adjusted to the animal, in order to record the reduction in temperature of the erythrocytes.

The protocols which follow do not give the absolute temperatures of the two parts, but rather the differences in temperature between the areas, as read directly from the galvanometer.

*Experiment 1.*—3 days after fracture of the left femur. At 2.40 p.m. one electrode was introduced into the inflamed area and the other into the corresponding area of the other limb.

| Time. | Difference in temperature between inflamed and healthy areas. | Time. | Difference in temperature between inflamed and healthy areas. |
|---|---|---|---|
| *p. m.* | °C. | *p. m.* | °C. |
| 2.48 | 0.8 | 4.45 | 3.0 |
| 2.49 | 0.9 | 4.55 | 3.0 |
| 2.55 | 0.8 | 5.10 | 2.6 |
| 3.05 | 0.7 | 6.15 | 1.9 |
| 3.18 | 0.9 | 6.30 | 1.7 |
| (Killed by puncturing the medulla.) | | 6.40 | 1.7 |
| 3.21 | 1.3 | 6.50 | 1.6 |
| 3.24 | 0.9 | 7.05 | 1.4 |
| 3.30 | 1.0 | 7.15 | 1.4 |
| 3.37 | 1.9 | 7.30 | 1.3 |
| 3.45 | 2.3 | 9.35 | 0.9 |
| 3.53 | 2.7 | 10.05 | 0.6 |
| 4.00 | 3.0 | 10.35 | 0.5 |
| 4.10 | 3.1 | 10.55 | 0.5 |
| 4.18 | 3.2 | 11.00 | 0.5 |
| 4.40 | 3.1 | After 10.00 a.m. the following day. | 0.2 |

In the foregoing experiment the difference of the initial temperature of the inflamed area and the healthy area is seen to vacillate between 0.7° and 0.9°C. When the circulation of the blood is arrested because of the death of the guinea pig, the difference in temperature increases considerably. After 17 minutes it was 1.9°C. and after an hour 3.2°. This progressive derangement in the equilibrium of the post-mortem temperature of the two parts under the same conditions

may be considered as being due to slow cooling or to the persistence of a longer isothermal activity in the warmer area.

The hypothesis of retardation in cooling which first presents itself might be admitted as true if it were possible to demonstrate greater persistence of activity in the inflamed area, but this hypothesis cannot be readily advanced in this special case where the volumes and surfaces of the parts were small and equal in sensitivity. On the other hand, the hypothesis cannot be excluded, inasmuch as the surrounding temperature was low and the temperature of the body as a whole did not control the rate of cooling.

A reasonable hypothesis appears to be the assumption that the accentuation and maintenance of the differences of temperature stand in relation to differences of isothermal, biochemical activity of the two parts. In the inflamed area we have actively proliferating tissues. It is probable that the local life of the inflamed tissues continues for some time after circulatory and respiratory death has occurred, and that there is an emission of heat which differs according to the initial activities of the affected tissues. The difference in temperature remains high for $1\frac{1}{2}$ hours and then diminishes gradually, but it was still 0.5°C. after 8 hours.

But the fact which is particularly interesting for this work is the variation of the initial temperature and the increase of the difference between corresponding areas which takes place in a few minutes. This increase tends to confirm the principle first laid down; *i.e.*, that as the circulation of a volume of liquid at uniform temperature ceases, the local temperature becomes apparent. This in the case under consideration would be different; *i.e.*, higher in the inflamed zone than in the corresponding healthy area.

The following experiments elucidate these points.

*Experiment 2.*—Fracture of the left femur, 4 days before. Surrounding temperature 39.5°C. At 10.25 a.m. one electrode was introduced into the inflamed area and the other into the corresponding area of the other side. A bolometer was applied to the thorax.

| Time. | Difference in temperature between inflamed and healthy areas. | Temperature of guinea pig. | Time. | Difference in temperature between inflamed and healthy areas. | Temperature of guinea pig. |
|---|---|---|---|---|---|
| *a.m* | °C. | °C. | *p. m.* | °C. | °C. |
| 10.35 | 1.2 | 39.0 | 12.10 | 3.2 | 27.8 |
| 10.40 | 1.2 | 39.0 | 12.17 | 3.0 | |
| 10.45 | 1.2 | 39.0 | 12.30 | 3.0 | |
| 10.47 | (Killed.) | | 12.35 | 2.7 | |
| 10.49 | 2.2 | | 1.30 | 2.0 | |
| 10.50 | 2.6 | 39.0 | 1.55 | 1.9 | |
| 10.51 | 2.6 | | 2.25 | 1.5 | |
| 10.53 | 2.8 | 38.6 | 2.55 | 1.4 | |
| 10.55 | 3.0 | 38.6 | 3.15 | 1.2 | |
| 10.57 | 3.0 | 38.0 | 3.40 | 1.0 | |
| 11.00 | 3.1 | | 4.20 | 0.9 | |
| 11.13 | 3.4 | 37.0 | 5.07 | 0.9 | |
| 11.32 | 3.4 | 33.4 | 5.50 | 0.8 | |
| 11.44 | 3.2 | | 11.35 | 0.3 | 15.5 |
| 11.51 | 3.2 | | | | 13.2 |

The experiment was conducted under the same conditions as those of Experiment 1.

During life the difference in temperature between the inflamed and the healthy zones was 1.2°C.; immediately after the death of the animal at 10.47 a.m. the difference increased. At 10.50 a.m., *i.e.* approximately 2 minutes after death, when the temperature of the bolometer was not sensibly altered, the local difference rose to 2.6°; *i.e.*, 1.4° more than during life. At 10.55 a.m., 5 minutes later, when the temperature of the body had fallen 0.4°, the difference between the two areas was 3°.

In this case we must exclude, I think, the idea that the causative factor is slower cooling, because the phenomenon manifests itself with a rapidity incompatible with that hypothesis. The temperature of the inflamed area was considerably higher than was apparent. The fact is demonstrated immediately when the disturbing influence of the blood which tends to carry off heat is no longer present.

The course of Experiment 2 corresponds approximately to that of the preceding experiment. The highest degree of difference was attained in a shorter period (after an hour, 3.4°C.), and it is noteworthy that even after 13 hours the difference was 0.3°.

In the preceding experiment we introduced a new element, the determination of the body temperature. It was shown that after a few minutes the general temperature had decreased 0.4°, while the difference between the local centers had increased 3°. This led me to consider it advisable to reduce the eventual causes of error consequent to external radiation. Experiment 3 was carried out in a room having a high and constant temperature (36° C.).

*Experiment 3.*—The guinea pig was placed in the calorimetric room which was kept at a temperature of 36°C. by means of electric lamps. A pair of batteries was connected with the fractured area and another pair with the corresponding healthy femur. The animal was provided with a bolometer.

| Time. | Difference in temperature between inflamed and healthy areas. | Temperature of guinea pig. | Room temperature. |
|---|---|---|---|
| *p.m.* | °C. | °C. | °C. |
| 2.20 | 3.1 | 39.4 | 36.0 |
| 2.25 | 3.1 | | 36.0 |
| 2.27 | 3.1 | 39.4 | 36.0 |
| | (Killed by puncturing the medulla.) | | |
| 2.31 | 4.6 | 39.1 | 36.0 |
| 2.40 | 4.6 | 39.0 | 36.0 |
| 2.47 | 3.6 | 39.0 | 36.0 |
| 2.50 | 3.0 | | |
| 2.55 | 2.6 | | |
| 3.03 | 2.1 | 39.0 | 35.8 |

In this manner, as the difference in the temperature of the guinea pig and that of the room was at a minimum, the curve of postmortem cooling should have had a slow course. From 2.28 p.m., when the animal was killed, until 2.30 p.m. the general temperature diminished 0.3°, and the difference between the local temperature of the inflamed area and the healthy zone increased 1.5°. A new fact is here brought out; *i.e.*, the markedly lessened permanence of the postmortem increase of thermal inequality between the two parts, the inflamed and the healthy areas.

This experiment does not give sufficient data to explain the fact,

but it should be noted that the initial difference in temperature of the inflamed center and the corresponding area was high, even before the guinea pig was killed, being about double the average. It is probable that the high room temperature which diminished the possibility of external radiation and kept the part warm, prevented the cooling effect of the blood. On the other hand, it is not excluded that the surrounding temperature itself may have caused some modification of the biochemical activity of the center by intensifying its activity.

To confirm and control the results that have been cited, other experiments were carried out. These consisted of experiments in which the circulation was interrupted permanently by the death of the animal and temporarily by local ischemia produced by compressing the abdominal aorta. In the latter case, it is possible to repeat the experiment a number of times and to modify it in details, thus by degrees neutralizing the errors of observation.

*Experiment 4.*—Fracture of the femur. The animal was placed in position at 1.30 p.m. Compression of the abdominal aorta at 3.10 p.m. and 3.21 p.m.

| Time. | Difference in temperature between inflamed and healthy areas. | Time. | Difference in temperature between inflamed and healthy areas. |
|---|---|---|---|
| *p. m.* | °C. | *p. m.* | °C. |
| 1.30 | 1.5 | 3.12 | 3.0 |
| 2.10 | 1.4 | 3.20 | 1.5 |
| 2.40 | 1.7 | | (Ischemia for 2 min.) |
| 3.00 | 2.0 | 3.23 | 4.0 |
| 3.10 | 2.0 | 3.40 | 1.7 |
| | (Ischemia for 2 min.) | 3.50 | 1.5 |

The experiment could not be more convincing. Immediately following the ischemia the difference in temperature of the two parts became accentuated, but it returned to the initial value or somewhat below it as soon as the normal circulation of blood was restored. The experiment cannot, however, be considered perfect, because the determination of the differences was not made during the ischemia, but immediately afterwards.

*Experiment 5.*—Fracture of the femur 5 days before. Conditions the same as in other experiments.

| Time. | Difference in temperature between inflamed and healthy areas. | Time. | Difference in temperature between inflamed and healthy areas. |
|---|---|---|---|
| *a. m.* | °C. | *p. m.* | °C. |
| 10.10 | 1.8 | 2.20 | 1.2 |
| 10.30 | 1.7 | | (Beginning of ischemia.) |
| 10.50 | 1.8 | 2.21 | 1.5 |
| | (Beginning of ischemia.) | 2.22 | 2.1 |
| 11.08 | 2.9 | 2.23 | 2.5 |
| 11.10 | 3.1 | 2.25 | 3.5 |
| 11.12 | 3.4 | | (Cessation of ischemia.) |
| | (Cessation of ischemia.) | 2.27 | 1.2 |
| 11.14 | 1.9 | 2.45 | 1.2 |
| 11.20 | 1.8 | | |

This experiment is a repetition of the preceding one, but marks an improvement in that readings were made during the ischemia; in addition, the second trial did not immediately follow the first, but was separated from it by an interval of 3 hours. An interesting point for further study is the fact that the initial difference in temperature of the inflamed and healthy areas amounts to 1.8°, while after 4 hours it is 1.2°. During the whole period when the thermoelectric battery remained in place, it probably initiated local injuries, and it is possible that these might have been the cause of an increased production of local heat.

The experiments reported above are supported and controlled by other analogous trials which are not given here because of the limitation of space. The experiments establish the fact that the difference in temperature of the inflamed and the corresponding healthy area increases rapidly and in a notable degree when the afflux of blood ceases because of a temporary stopping of the circulation or by a definite anemia. In view of these experiments made in an environment with a high temperature and taking into account for comparison the temperature of the body in relation to the time, we have excluded the possibility that the phenomenon depends upon retarded radiation of heat.

Because of the rapidity with which the phenomenon manifests itself, it does not seem probable that it could be related to a sudden

and more intense biochemical function of the inflamed cells stimulated to activity by an asphyxial condition.

It is consequently not the blood which heats the inflamed area, but, on the other hand, this area is kept by the active local hyperemia at a lower temperature than it would otherwise have. The finding which is apparently paradoxical is in fact true physiologically. In addition to the results cited by Cavazzani in his experiments on the liver, in muscle during contraction the venous blood is warmer than the arterial blood. The same is true of the parotid gland. Burton-Opitz (11) and Lefèvre speak of an equalizing temperature action of the circulation. In inflammation as well as in muscular contraction we have a notable local chemical activity. The circulation of the blood in both cases exercises its normal function, with the tendency of equalizing the temperature of the various areas.

The problem might be considered complete at this stage, but at the suggestion of Professor Grandis a more detailed study of the process was made, in order to establish if possible under what special conditions calorification develops and is influenced.

In the experiments made at a high room temperature it had been shown that the disturbance in thermal equilibrium of the corresponding areas was less permanent than at a low room temperature. Hence it seemed worth while to try the partial saturation of the organism with carbon dioxide to diminish the intake of oxygen into the tissues, or poisoning by cyanide of potassium or chloral to inhibit the local processes of oxidation. This series of studies, however, did not always lead to concrete results on account of the difficulty of attaining the required degree of saturation of the organism with the substances employed before death occurred. The experiments made with illuminating gas and cyanide of potassium failed to give convincing results, because death occurred at an early stage, when it was not probable that the toxic substances had been uniformly diffused in the organism so as to give the necessary degree of saturation. The experiments are therefore omitted. The results were better with chloral.

*Experiment 6.—*

| Time. | Difference in temperature between inflamed and healthy areas. | Remarks. |
|---|---|---|
| *a.m.—p.m.* | °C. | |
| 10.50 | 2.0 | |
| 11.20 | 1.8 | |
| 11.50 | 1.8 | |
| 12.30 | 1.8 | |
| 2.00 | 1.2 | |
| | (Beginning of ischemia.) | |
| 2.10 | 3.1 | |
| 2.15 | 1.4 | |
| | (Cessation of ischemia.) | |
| 2.20 | 1.5 | |
| | (Chloral administered.) | |
| 2.40 | 1.1 | Animal alive. |
| 2.45 | 1.2 | " " |
| 3.00 | 1.2 | " " |
| 3.20 | 1.0 | Heart beating. |
| 3.35 | 0.8 | Seems dead. |
| 3.40 | 0.8 | Animal " |
| 4.00 | 0.7 | |

This experiment confirms in the first place a diminution of difference in temperature of an inflamed and a sound limb after some hours of irritation of the healthy part. It also confirms the fact initially demonstrated, that the temporary ischemia always augments considerably and with great rapidity the disproportion of temperature of the parts. In addition, it establishes the fact that by administering a strong dose of chloral such as kills a guinea pig in a period varying from an hour to less than 80 minutes, there is an absence of the phenomenon normally observed when the guinea pig is killed by puncture of the medulla.

During the entire period in which the chloral manifested its toxic action and particularly in the interval between 3.20 p.m., when the guinea pig was certainly alive, and 3.40 p.m., when the animal was undoubtedly dead, the difference in temperature of the healthy and the inflamed areas did not increase; on the contrary, it showed a tendency to diminish. It is well known that chloral exercises an inhibiting effect on organic cellular oxidations (15). Even if my statements were not confirmed by the extensive literature on the subject,

which shows that there is a diminution of the carbon dioxide emitted and a lessened consumption of oxygen by the respiratory change, the suggestive experiments on the emission of heat before death, which diminishes with increased doses of chloral,[2] would be convincing.

These experiments, therefore, seem to indicate that the increase in the temperature of the inflamed area is due to a local cellular hyperfunction, for when this cellular activity is paralyzed the increase does not occur.

## SUMMARY.

The experiments set forth here establish the fact that the heat of the inflamed part has its origin primarily in the local biochemical activity of the cellular elements which participate in the inflammatory process. The inflammatory hyperemia, instead of being the necessary and constant source of the inflammation must be considered a natural physiological compensation for the abnormal local calorification.

The rapid circulation of the blood in the inflamed part tends to moderate the increase in local temperature and to equalize the temperature with that of other parts of the body.

## BIBLIOGRAPHY.

1. Galeotti, in Lustig, A., Patologia generale, ii, 152. Lubarsch, O., in Aschoff, L., Pathologische Anatomie, Jena, 3rd edition, i, 514.
2. Cohnheim, in Lustig, A., Patologia generale, i, chap. v.
3. Jacobson, H., *Virchows Arch. path. Anat.*, 1870, li, 275.
4. Bernard, C., *Centr. med. Wissensch.*, 1869, vii, 861.
5. Schneider, *Med. Centr.*, 1870.
6. Huppert, M., *Arch. Heilk.*, 1873, xiv, 73.
7. Liebermeister, C., Das Fieber, Leipsic, 1875, 375.
8. Cohnheim (2), p. 187.
9. Cf. Weber, O., in von Pitha and Billroth, Handbuch der allgemeine und specielle Chirurgie, Erlangen, 1865, i, pt. 1; Galeotti, in Lustig, A., Patologia generale, ii, 153.

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[2] These experiments have been published in a series of thermocalorimetric studies on the last periods of life, *Accad. Lincei*, 1913, xxii, 76. From the experiments it was found that the moment of thermal death precedes by a certain interval the cardiac and respiratory deaths.

10. Ludwig, C., and Spiess, A., *Sitzungsb. k. Akad. Wissensch.*, 1857.
11. Burton-Opitz, R., *Arch. ges. Physiol.*, 1903, xcvii, 309.
12. Courmont, J., Traité de pathologie général, Paris, 1900, iii, 391.
13. Bernard, C., Recherches expérimentales sur le grand sympathique et spécialement sur l'influence que la section de ce nerf excerce sur la chaleur animal, Paris, 1854, 281.
14. Kussmaul, A., and Tenner, A., in Moleschott, J., Untersuchungen zur Naturlehre des Menschen und der Thiere, Frankfort-on-Main and Giessen, 1857, iii, 1, cited by Recklinghausen, p. 205.
15. Guinard, L., in Richet, C., Dictionnaire de physiologie, Paris, 1898, iii, 551. Eulenburg, A., Real encyclopædia, 4th edition, 1908.

# A STUDY OF ATYPICAL TYPE II PNEUMOCOCCI.

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Study of the serological relation of strains of pneumococcus has shown that this organism may be classified by means of biological reactions into distinct types. From the first studies made it was evident that there existed three types of pneumococcus, the individual members of each type being alike in their immune reactions, the types themselves, however, differing sharply from one another. These types are now commonly known as Types I, II, and III. In addition to these fixed types there remain an indeterminate number of unrelated strains which for convenience have been grouped together and constitute the heterogeneous Type IV. More minute study of the last group indicates that it in turn can be further separated into a number of closely related immunological types.

The classification of pneumococcus was originally based upon immunological differences brought out by the reaction of protection. It later developed that the reaction of agglutination was as specific as the more time-consuming protection test, and recognition of this fact has led to its adoption as a routine method. In the course of the study of a large number of strains isolated from lobar pneumonia, it was observed that certain of these agglutinated atypically in Type II serum. Study of this phenomenon revealed the fact that these atypical Type II organisms possess partial antigenic characters common to the Type II pneumococcus, but vary from the typical representatives of this type by a diversity of relations among themselves, and by a lack of the reversibility of their immune reactions with the type organism. Because of these variations these organisms have been classified by Avery[1] as subvarieties of Pneumococcus Type II. In a study of ten such strains it was found possible to classify them

[1] Avery, O. T., *J. Exp. Med.*, 1915, xxii, 804.

into three distinct subtypes, IIa, IIb, and IIx. By means of agglutination, absorption, and protection experiments the members of Subtype IIa and IIb were found to possess immunity reactions identical with all other strains of the homologous types. Subtype IIx, however, consisted of a heterogeneous series of independent strains which showed neither cross-agglutination nor cross-protection.

The study reported in this paper was undertaken in order to determine the occurrence and frequency of atypical Type II pneumococci and their relation to lobar pneumonia, and to elaborate further their classification on the basis of their specific relations.

204 strains of pneumococci, all of which showed atypical agglutination in Type II serum, were studied. 77 strains were obtained from lobar pneumonia, 5 from postoperative pneumonia, 1 from meningitis, 5 from guinea pig pneumonia, 100 from normal mouths, 6 from convalescent Type I pneumonia, 3 from convalescent Type II pneumonia, 1 from convalescent Type III pneumonia, and 6 from dust.

In classifying these strains, agglutination and absorption reactions have been employed. In the agglutination reactions equal parts of broth cultures and immune sera were used, except where dilutions of sera were employed, when 0.1 cc. of culture in 0.9 cc. of immune serum diluted in normal salt solution was used. The agglutinations were incubated 2 hours in a water bath at 37°C., kept in the ice box over night, and readings made the next morning.

On the basis of specific agglutination in monovalent rabbit sera, the 204 strains were classified into twelve distinct groups as shown in Table I. From Table I it is seen that Subgroup IIb is the largest, representing 30 per cent of all strains studied. Next in order of frequency come Subgroups IIa, IIc, and IIm. These four groups comprise 73 per cent of all the strains classified. Subgroups IIa and IIb correspond to Avery's IIa and IIb, while the three strains originally placed in the IIx group were in the study of this series finally classifiable into Subgroups IIj, IIg, and IIe.

Only two strains showed cross-agglutination in the immune sera of a heterologous group. Strain St. 21 agglutinated promptly in serum of Subgroup IIm and slowly in that of Subgroup IIk. This culture was agglutinated by Serum IIm in a dilution of 1:160, but

only in a dilution of 1 : 80 by Serum IIk. A rabbit was immunized to Strain St. 21, and the type cultures, Nos. IIm and IIk, were tested for agglutinability in this serum.

From Table II it is seen that Culture IIm is agglutinated by Serum St. 21 in dilution of 1 : 300, while Culture IIk is affected by this serum only in a dilution of 1 : 100. Serum St. 21 was then exhausted

TABLE I.

*Classification of 204 Strains of Type II Atypical Pneumococci.*

| Type. | Incidence. | |
|---|---|---|
| | | *per cent* |
| IIa | 38 | 19 |
| IIb | 62 | 30 |
| IIc | 28 | 14 |
| IId | 11 | 5 |
| IIe | 4 | 2 |
| IIf | 12 | 6 |
| IIg | 3 | 1 |
| IIh | 7 | 3 |
| IIj | 3 | 1 |
| IIk | 5 | 2 |
| III | 11 | 5 |
| IIm | 20 | 10 |

TABLE II.

*Agglutination of Cultures IIk and IIm in St. 21 Serum.*

| Culture No. | Dilution of Serum St. 21. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 : 10 | 1 : 20 | 1 : 40 | 1 : 80 | 1 : 100 | 1 : 160 | 1 : 200 | 1 : 240 | 1 : 300 | Control. |
| IIk | ++ | ++ | ++ | + | + | − | − | − | − | − |
| IIm | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | − |
| St. 21 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | − |

of agglutinins by absorption with Culture IIk, and its agglutinin titer determined for Cultures St. 21 and IIm.

From Table III it is seen that absorption of St. 21 sera with Culture IIk does not materially affect the agglutinins for Cultures St. 21 and IIm. Another lot of St. 21 sera was absorbed with Culture IIm. The results are shown in Table IV. From Table IV it is seen that

absorption of St. 21 serum with Culture IIm removes the agglutinins for both Strains St. 21 and IIk.

Strain P 65, which also showed cross-agglutination in heterologous sera, reacted slowly in Serum IIc and promptly in Serum IIm. This strain was tested like No. St. 21 by the absorption method and likewise showed that the reaction in Serum IIc was attributable to a minor agglutinin.

TABLE III.

*Agglutination of Cultures St. 21 and IIm in St. 21 Serum Exhausted of Agglutinins for Strain IIk.*

| Culture No. | Dilution of Serum St. 21. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 : 10 | 1 : 20 | 1 : 40 | 1 : 80 | 1 : 100 | 1 : 160 | 1 : 200 | 1 : 240 | 1 : 300 | Control. |
| IIk | – | – | – | – | – | – | – | – | – | – |
| IIm | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | – |
| St. 21 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | + | + | – |

TABLE IV.

*Agglutination of Cultures St. 21 and IIk in St. 21 Serum Exhausted of Agglutinins for Strain IIm.*

| Culture No. | Dilution of Serum St. 21. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 : 10 | 1 : 20 | 1 : 40 | 1 : 80 | 1 : 100 | 1 : 160 | 1 : 200 | 1 : 240 | 1 : 300 | Control. |
| IIk | – | – | – | – | – | – | – | – | – | – |
| IIm | – | – | – | – | – | – | – | – | – | – |
| St. 21 | – | – | – | – | – | – | – | – | – | – |

Table V shows the source and type of the 204 strains studied. 77, or 38 per cent, were from cases of lobar pneumonia, 5 from post-operative pneumonia, and 1 from meningitis. 4 of the 5 strains from epidemic pneumonia in guinea pigs were isolated from guinea pigs in one city, while the 5th strain was from an epidemic in a different locality. All the guinea pig strains belonged to the same group. It has been shown that during convalescence from pneumonia the disease-producing types of pneumococci tend to disappear and are often replaced by Type IV or atypical Type II pneumococci, which have been previously shown to be the types most commonly found in

normal mouths. During convalescence the saliva of 6 Type I, 3 Type II, and 1 Type III patients showed atypical Type II pneumococci. If these are included, 110, or more than one-half of all the strains studied, may be considered as inhabitants of the normal mouth. 6 strains were recovered from dust.

From an examination of this table it is seen that the group may be divided according to the frequency of occurrence into two classes: (1) those found predominantly in disease; (2) those which occur more frequently in normal mouths.

TABLE V.

*Source and Type of Atypical Type II Pneumococci.*

| Source. | Type. | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | e | f | g | h | j | k | l | m | Total. | |
| | | | | | | | | | | | | | | *per cent* |
| Lobar pneumonia | 33 | 12 | 2 | 5 | 1 | 2 | 1 | 7 | 2 | 2 | 6 | 4 | 77 | 38 |
| Postoperative pneumonia | 1 | 2 | | | 1 | | | | | | | 1 | 5 | 2 |
| Meningitis | | | | | | | | | | | | 1 | 1 | 0.5 |
| Guinea pig pneumonia | | | 5 | | | | | | | | | | 5 | 2 |
| Normal mouths | 2 | 38 | 18 | 6 | 2 | 10 | 2 | | 1 | 2 | 5 | 14 | 100 | 49 |
| Convalescent Type I | 2 | 1 | 2 | | | | | | | 1 | | | 6 | 3 |
| " " II | | 3 | | | | | | | | | | | 3 | 1 |
| " " III | | | 1 | | | | | | | | | | 1 | 0.5 |
| Total normal mouths | 4 | 42 | 21 | 6 | 2 | 10 | 2 | 0 | 1 | 3 | 5 | 14 | 110 | 53 |
| Dust | | 6 | | | | | | | | | | | 6 | 3 |
| Total | 38 | 62 | 28 | 11 | 4 | 12 | 3 | 7 | 3 | 5 | 11 | 20 | 204 | |

In the disease-producing class of atypical Type II organisms belong Subgroup IIa, with 33 strains out of a total of 38 from pneumonia, and Subgroup IIh, all of whose 7 strains are from pneumonia. Organisms of Subgroups IIb, IIc, IIf, and IIm are found frequently in healthy mouths, although they may also be found in association with disease. In some instances the groups comprise so few strains that it is impossible to determine their relation in either of these two classes.

During the last 3 years pneumococci have been isolated in 458 instances from 526 cases of lobar pneumonia admitted to the wards of

the Hospital of The Rockefeller Institute. These organisms have been typed according to the biological classification described by Dochez and Gillespie.[2] Of the 458 strains[3] obtained from disease, 52, or 11 per cent, were found to be atypical Type II organisms. Clough[4] in a study of 121 strains of pneumococci isolated from disease found 22, or 18 per cent, atypical Type II organisms. Sydenstricker and Sutton[5] in a study of 150 healthy individuals recovered atypical Type II pneumococci in 5 instances, and in 62 cases of pneumonia found this organism present in 12. They report a mortality

TABLE VI.

*Type Incidence and Mortality of 59 Atypical Type II Pneumonias.*

| Type. | No. of cases of pneumonia. | Incidence. | No. of deaths. | Mortality. |
|---|---|---|---|---|
| | | *per cent* | | *per cent* |
| IIa | 23 | 38 | 8 | 35 |
| IIb | 10 | 17 | 1 | 10 |
| IIc | 1 | 1 | 0 | 0 |
| IId | 5 | 8 | 3 | 60 |
| IIe | 1 | 1 | 0 | 0 |
| IIf | 1 | 1 | 0 | 0 |
| IIg | 0 | 0 | 0 | 0 |
| IIh | 7 | 12 | 4 | 57 |
| IIj | 2 | 3 | 0 | 0 |
| IIk | 1 | 1 | 0 | 0 |
| III | 5 | 8 | 1 | 20 |
| IIm | 3 | 5 | 1 | 33 |
| Total..... | 59 | | 18 | |

of 31 per cent in pneumonia associated with atypical Type II pneumococci.

Table VI gives the mortality of 59 cases of lobar pneumonia from which atypical Type II pneumococci were isolated. 45 of these cases were in the Hospital of The Rockefeller Institute, 12 in the Presby-

[2] Dochez, A. R., and Gillespie, L. J., *J. Am. Med. Assn.*, 1913, lxi, 727.

[3] Stillman, E. G., *J. Exp. Med.*, 1917, xxvi, 519.

[4] Clough, M. C., *Bull. Johns Hopkins Hosp.*, 1917, xxviii, 306.

[5] Sydenstricker, V. P. W., and Sutton, A. C., *Bull. Johns Hopkins Hosp.*, 1917, xxviii, 312.

terian Hospital, and 2 were not hospital cases. Although these figures are too small to warrant any conclusion, the total mortality of 32 per cent indicates a high pathogenicity and virulence. The three subgroups, IIa, IId, and IIh, showed the highest mortality.

## DISCUSSION.

Avery, working with only ten strains, was able to differentiate two definite subgroups, IIa and IIb. In the present study of 204 strains, ten other groups have been recognized. It is possible that if a larger series were studied, still other groups would be found. It is significant, however, that all of the 204 strains were classified in one or another of these twelve groups. Only two strains showed cross-agglutination in the immune sera of a heterologous group and these reactions were shown to be due to the presence of minor agglutinins for one group. Indeed it is surprising that the specificity of these groups is as definite as it is.

Although atypical Type II pneumococci are found associated with about 11 per cent of cases of lobar pneumonia, they have an incidence of 18.3 per cent in normal mouths. But as certain subgroups, IIa and IIh, are encountered more frequently in relation to disease, it is probable that they have a greater pathogenicity. These same groups occur rarely in normal mouths. Just the opposite is the case with Subgroups IIb, IIc, IIf, and IIm, which are much more frequently encountered living a saprophytic existence in normal mouths than in association with disease. Type I and II pneumococci cause a large percentage of pneumonia, but occur rarely in normal mouths. On the other hand, the Type III and IV organisms, although occasionally found in association with pneumonia, occur frequently in normal mouths. Similarly the atypical Type II pneumococci exhibit the same division into parasitic and saprophytic types as is already recognized in the major classification.

## SUMMARY.

1. At least twelve subgroups of atypical Type II pneumococcus may be recognized by specific agglutination reactions. They have been designated Subgroups IIa, IIb, IIc, IId, IIe, IIf, IIg, IIh, IIj, IIk, IIl, and IIm.

2. These subgroups have an incidence of 11 per cent in lobar pneumonia, and of 18 per cent in normal mouths.

3. Certain groups, IIb, IIc, IIf, and IIm, occur in normal mouths.

4. Subgroups IIa and IIh are met with largely in connection with disease.

5. The mortality of acute lobar pneumonia due to these atypical Type II pneumococci is fairly high—32 per cent.

The author acknowledges his indebtedness to Miss Miriam P. Olmstead of the Presbyterian Hospital for 41 of the cultures of pneumococci used in this study.

# THE OCCURRENCE OF BACILLUS INFLUENZÆ IN THROATS AND SALIVA.

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(*From the Hospital of The Rockefeller Institute for Medical Research.*)

Plates 10 to 13.

(Received for publication, January 15, 1919.)

In the present paper are reported the facts obtained during an investigation of the bacteriology of influenza which included (1) the use of oleate hemoglobin agar, (2) the incidence of *Bacillus influenzæ* in uncomplicated and complicated cases of influenza and the associated types of pneumococci, (3) the occurrence of *Bacillus influenzæ* in convalescents, and (4) the occurrence of *Bacillus influenzæ* in the mouth secretions or throats of normal people.

The medium used in this work was Avery's oleate hemoglobin agar.[1] Agar of an average hydrogen ion concentration of 7.2 was used. As the domestic sodium oleate which was tested from time to time gave less consistent results, Kahlbaum's sodium oleate was used. This medium is especially favorable to the growth of Gram-negative organisms in influenza work. On these plates pneumococci do not grow and streptococci rarely grow, although some staphylococci show a scant growth (Figs. 1, 2, and 6). After 36 to 48 hours incubation several types of *Bacillus influenzæ* colonies are encountered on this medium. The characteristic colony of *Bacillus influenzæ* was clear, round, slightly glistening, convex, and discrete. The size varied from pin-point colonies, easily confused with diphtheroids or *catarrhalis* colonies, to large round colonies of 1 to 3 mm. in diameter. The larger characteristic colonies had a distinct nucleus which was much darker than the rest of the colony (Figs. 3, 4, and 7). The colonies were very sticky in consistency and would streak across the agar and follow the loop in long threads. The medium beneath the colony appears discolored after a colony is fished.

[1] Avery, O. T., *J. Am. Med. Assn.*, 1918, lxxi, 2050.

The colonies most readily confused on inspection with those of *Bacillus influenzæ* were those of *Micrococcus catarrhalis*, meningococcus, diphtheroids, and especially an unidentified organism called "Bacillus X." This organism was encountered in about two dozen individuals. The colony varied between 1 and 2 mm. in diameter, was clear, convex, and mucoid. The nucleus so common in the influenza colony was absent, but the colony left a slight discoloration on the agar. This bacillus is Gram-negative, takes the counterstain deeply, and when taken from agar surface cultures appears as long tangled threads, somewhat similar to the threads often seen in pure cultures of *Bacillus influenzæ*. In blood broth cultures it appears as a small fat bacillus without chain formation. It does not grow on plain sheep serum, or glucose agar. On blood agar the abundant growth appears as a clear, somewhat granular, fairly heavy film, with marked hemolysis of the red cells. This hemolysis is as marked as that produced by hemolytic streptococci (Fig. 5). Complete hemolysis is produced in blood broth after 24 hours incubation. After several days both blood broth and blood agar cultures turned brown and then blackish from the formation of methemoglobin. After 7 days incubation, litmus milk, to which a little blood had been added, showed an alkaline reaction with peptonization. No pathogenicity could be demonstrated for mice, rats, or rabbits.[2]

In all the cultures from convalescents and normal persons, and in many from the hospital patients, the organism was isolated in pure culture and was demonstrated to possess the typical morphology and shown to be strictly hemoglobinophilic. All evidence based simply

[2] The other organisms commonly found in cultures from the throat on this medium may be briefly described as follows in the frequency of their occurrence. (1) Gram-negative cocci of the *Micrococcus catarrhalis* group. The colonies are round, sharply defined, with a ridged or lined surface. Some colonies are so scaly that they lift off the medium as a pellicle; other colonies of this group will slide across the surface of the agar without breaking. (2) Staphylococci. Minute, pin-point colonies. (3) Diphtheroids. Minute, pin-point colonies. (4) Meningococci. The colonies are clear, round, and discrete. (5) Friedländer's bacillus. The colonies are white, mucoid, and occasionally confluent.

Occasionally in some plates a few Gram-positive streptococci may grow. These colonies are minute, dense, nucleated, and sharply defined. Pneumococci were never found and "spreaders" rarely grow.

on the presence of slender Gram-negative bacilli in films was discarded as of no value. The wide variations in the morphology of *Bacillus influenzæ* noted by Wollstein[3] were observed in this series. This study was made during the widespread epidemic of influenza which occurred in New York. The epidemic began about the middle of September, reached its height about the middle of October, and then gradually fell, though cases are still present in the city.

In Table I are shown the incidence of *Bacillus influenzæ* and the type of pneumococcus recovered from the patients admitted to the Hospital of The Rockefeller Institute, suffering from influenza,

TABLE I.

*Incidence of B. influenzæ and Types of Pneumococci in Influenza and Pneumonia.*

| Disease. | No. of cases. | *B. influenzæ.* | | Type pneumococcus. | | | | No. of deaths. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | I | II | III | IV | | |
| | | | *per cent* | | | | | | |
| Uncomplicated influenza. | 49 | 41 | 83+ | 0 | 2 | 2 | 17 | 0 | |
| Influenza, with bronchopneumonia. | 43 | 40 | 93 | 1 | 0 | 3 | 27 | 12 | One case, Pneumococcus Types III and IV. |
| Bronchopneumonia. | 6 | 6 | 100 | 0 | 0 | 1 | 4 | 0 | |
| Lobar pneumonia. | 20 | 11 | 55 | 3 | 2 | 6 | 7 | 7 | One case, Friedländer's bacillus; one, staphylococcus. |
| Total..... | 118 | 98 | 82 | 4 | 4 | 12 | 55 | 19 | |

bronchopneumonia, and lobar pneumonia during the 14 weeks between September 16, and December 31, 1918. In these cases of influenza and pneumonia the presence of *Bacillus influenzæ* was determined in the majority of instances by blood agar plates and mouse inoculation. The selective medium, oleate hemoglobin agar, was used only in the latter part of the investigation.

From 49 cases of uncomplicated influenza *Bacillus influenzæ* was recovered in 41 instances, or 83 per cent. From the 43 cases which were complicated by bronchopneumonia this organism was cultivated

[3] Wollstein, M., *J. Exp. Med.*, 1915, xxii, 445.

in 40, or 93 per cent. The 6 cases of bronchopneumonia, which were probably late cases of influenza, all showed influenza bacilli. The incidence of *Bacillus influenzæ* is much lower in the 20 cases of lobar pneumonia, as only 11 cases, or 55 per cent, were positive.

The distribution of the types of pneumococci in these cases is especially interesting. Whereas normally pneumococci of Types I and II are found associated with over 60 per cent of the cases of lobar pneumonia, in the cases of bronchopneumonia complicating influenza they are rare in comparison with Type III and IV pneumococci, which are normally and commonly found in the mouths of healthy persons.

In order to determine how many normal persons and those convalescent from influenza harbor influenza bacilli, a study was made of the personnel of the Laboratories and the Hospital of The Rockefeller Institute and the War Demonstration Hospital. From each individual a throat culture was taken with a sterile swab, which was then rubbed across a corner of the oleate hemoglobin plate. A small amount of mouth saliva was also collected in a sterile dish from each case. A loopful of saliva was later placed on a side of the plate. Each plate was streaked with a loop according to the Sunburst method[4] (Figs. 8 and 9). The plates were examined for *Bacillus influenzæ* after an incubation period of from 36 to 48 hours at 37°C.

From Table II it is seen that of 54 convalescent cases, 25, or 46 per cent, harbored *Bacillus influenzæ*. The length of time that had elapsed between recovering from influenza and the date of the culture varied from 1 week to 4 months. The shortest period in which a convalescent had apparently ceased to be a carrier of *Bacillus influenzæ* was 1 week. The longest period during which a convalescent was known to carry *Bacillus influenzæ* was 3 months. The average carrying period, as fixed by the time of taking the cultures, was about 6 weeks. Of 177 persons who gave no history of having had influenza, 74, or 42 per cent, carried *Bacillus influenzæ*. The total incidence of influenza carriers among the 231 normals and late convalescent individuals was 99, or 43 per cent.

[4] Standard technique for meningococcus carrier detection; adopted by the Medical Department of the U. S. Army, U. S. Navy, and U. S. Public Health Service.

In the above cases both throat and saliva cultures were made on oleate plates.

In all the 231 cases here represented, 73 showed a positive culture of *Bacillus influenzæ* from a throat swab (Table III). In each instance only a single throat culture or sputum culture was taken. 55

TABLE II.

*Incidence of B. influenzæ in Convalescents and in Normal Individuals.*

| Individuals. | No. | Positive cases. | |
|---|---|---|---|
| | | | *per cent* |
| Convalescents | 54 | 25 | 46 |
| Normals | 177 | 74 | 42 |
| Total | 231 | 99 | 43 |

TABLE III.

*Comparison of Throat and Saliva Cultures.*

| Individuals. | Throat +; saliva −. | Throat −; saliva +. | Throat +; saliva +. | Throat −; saliva −. | Total. |
|---|---|---|---|---|---|
| Convalescents | 14 | 6 | 5 | 29 | 54 |
| Normals | 30 | 20 | 24 | 103 | 177 |
| Total | 44 | 26 | 29 | 132 | 231 |

TABLE IV.

*Incidence of Positive Carriers among Vaccinated and Non-Vaccinated Normal Individuals.*

| Vaccinated. | | Non-vaccinated. | | Vaccinated. | | Non-vaccinated. | |
|---|---|---|---|---|---|---|---|
| Negative. | Positive. | Negative. | Positive. | Total. | Positive. | Total. | Positive. |
| | | | | | *per cent* | | *per cent* |
| 30 | 16 | 73 | 58 | 46 | 35 | 131 | 44 |

positive cultures of *Bacillus influenzæ* were obtained from saliva. This would seem to indicate that throat cultures are on the whole preferable to saliva cultures as a means of isolating *Bacillus influenzæ* from normal individuals or late convalescents. It must, however, be pointed out that in 26 instances *Bacillus influenzæ* was recovered from the saliva only, the throat cultures being negative.

Of the 177 normal people studied, 46 had received influenza vaccine. Of these, 35 per cent were positive carriers as compared with an incidence of 44 per cent among the 131 non-vaccinated individuals (Table IV). These observations were based on single cultures in each case. Thus it is apparent that there is no striking relation between vaccination and the carrier state. The importance of our observations is greatly lessened by the fact that many of those vaccinated had received their vaccine elsewhere and were as a rule unable to give any accurate information as to the dosage used.

## DISCUSSION.

As a result of this study it is evident that during the influenzal epidemic period *Bacillus influenzæ* could be easily recovered from throats and saliva when a selective medium was used. The chief difficulty of isolating influenza bacilli from throats is analogous to that encountered in isolating meningococci from the nasopharynx; namely, that other organisms tend to overgrow *Bacillus influenzæ* just as they overgrow the delicate meningococci. The frequent occurrence of other Gram-negative bacilli, which morphologically may be easily confused with *Bacillus influenzæ*, renders the diagnosis of this organism from films alone valueless.

The high incidence of cultivation of *Bacillus influenzæ* from the upper respiratory tract of cases of influenza and bronchopneumonia during the epidemic is a point in favor of the view that this organism may be of significance in the disease in question. The fact that the incidence was high during this period in the cases of lobar pneumonia is not necessarily opposed to this view. Convalescent patients and normal individuals show about the same per cent of positive findings. It is noticeable that during the latter part of December, coincident with the ebb of the epidemic, the number of positive cultures decreased. The types of pneumococci found associated with influenza cases are of interest. Whereas normally 60 per cent of the cases of lobar pneumonia are associated with the presence of Type I and Type II pneumococci, these types are infrequently encountered in the pulmonary complications of influenza. On the contrary, the types which occur frequently in normal mouths, Types III and IV, are

usually recovered from the cases of bronchopneumonia which develop following influenza. At the same time a considerable number of the cases of lobar pneumonia occurring during this period were associated with the presence of pneumococci of Types I and II.

From this study it is clear that a diagnosis of *Bacillus influenzæ* is valueless if based only on the evidence obtained from direct films of saliva, throats, or cultures. It is also apparent that *Bacillus influenzæ* may be recovered in pure culture from a large percentage of acute cases of influenza, from convalescents, and from normal persons, if a suitable differential medium is used.

## CONCLUSION.

1. Oleate hemoglobin agar is a good selective culture medium for *Bacillus influenzæ*.

2. *Bacillus influenzæ* has been cultivated from the mouth of 93 per cent of cases of influenza and bronchopneumonia.

3. *Bacillus influenzæ* was present at the time of this study in the mouths of 43 per cent of normal individuals.

4. The types of pneumococci found associated with the complicating bronchopneumonias of influenza are the types which are usually found in normal mouths.

## EXPLANATION OF PLATES.

### Plate 10.

Fig. 1. Plain blood agar plate. The lateral sectors show growth of pneumococcus and *Streptococcus hæmolyticus*. The lower sector shows *B. influenzæ*; growth is present, but not visible in the photograph.

Fig. 2. Oleate hemoglobin agar plate. Corresponding sectors are planted with the same organisms as in Fig. 1 and show enhanced growth of *B. influenzæ* in the lower sector and complete inhibition of growth of pneumococcus and *Streptococcus hæmolyticus*.

### Plate 11.

Fig. 3. Pure culture of *B. influenzæ* on oleate hemoglobin agar showing large nucleated colonies after 36 hours incubation.

Fig. 4. Throat culture from a case of lobar pneumonia on oleate hemoglobin agar showing large nucleated colonies of *B. influenzæ* after 36 hours incubation.

Fig. 5. Blood agar plate showing hemolysis produced by hemolytic streptococcus (top) and that produced by "Bacillus X" (bottom).

PLATE 12.

FIG. 6. Growth of *B. influenzæ* on oleate hemoglobin agar; 15 hours growth at 37°C.

FIG. 7. Throat culture from a normal person on oleate hemoglobin agar showing large colonies of *B. influenzæ*; 36 hours growth at 37°C.

PLATE 13.

FIG. 8. Throat culture from a case of lobar pneumonia on oleate hemoglobin agar showing large colonies of *B. influenzæ*; 36 hours incubation at 37°C.

FIG. 9. Throat culture from a normal person on oleate hemoglobin agar showing many small colonies of *B. influenzæ;* 36 hours incubation at 37°C.

# THE DETERMINATION OF THE LENGTH OF LIFE OF TRANSFUSED BLOOD CORPUSCLES IN MAN.

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PLATE 14.

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Whether transfused blood corpuscles live and function for any considerable length of time, or whether the beneficial results that have been observed to follow transfusion, outside the purely mechanical part of increasing the bulk of the depleted blood, are due to a stimulating effect on the hemopoietic function by the product of the broken down corpuscles is still an open question (Archibald, Crile, Hunter, Kimpton, Primrose, Robertson and Watson).

Marfels and Moleschott (1856) and Brown-Sequard (1867) studied the question of the length of life of blood corpuscles by injecting nucleated corpuscles (bird and frog) into animals having non-nucleated corpuscles, and watching for the disappearance of the nucleated corpuscles from the circulation of the transfused animal. It is now known that these results have no bearing on the question, as it was not blood destruction that was being studied but the elimination of foreign protein. The same objection holds good for any attempt to stain blood corpuscles with so called vital stain and to reinject them, since there is no stain known that will hold without destroying the living quality of the corpuscles; therefore, in the stained corpuscle we are again dealing with a foreign body capable of more or less rapid removal than transfused blood.

Thus far the only satisfactory direct methods of attacking the problem of the length of life of transfused corpuscles have been those involving the study of the changes of the erythrocyte count following transfusion. Extensive work along this line, done on animals by Ward-Muller, Quinck, von Ott, and Hunter, was reviewed by Hunter in 1885 and 1886. Ward-Muller transfused dogs and produced

a plethora. 2 or 3 days afterward he found that the number of blood corpuscles corresponded closely to the number of the original corpuscles plus the transfused; a few days later the injected corpuscles, judged by the decrease in blood count, began to break down, and by the end of a few weeks all the injected corpuscles had disappeared. In order to produce the effect of a very slow transfusion Hunter injected blood intraperitoneally and found that the blood count increased up to the 2nd or 3rd day, after which it gradually decreased and reached normal from the 14th to the 26th day, when 40 to 90 per cent of the total blood volume was injected. Von Ott, in order to avoid the abnormal condition of plethora, removed from one-half to two-thirds of the blood from animals and replaced it with defibrinated blood. He noted a gradual fall in the blood count which reached a minimum from the 19th to the 22nd day. From then on an increase took place which reached normal 2 weeks later. The lowest part of this curve would not, of course, represent the last of the transfused blood corpuscles but the point after which their destruction was surpassed by the regeneration of new corpuscles. The time that elapsed before the complete disappearance of transfused blood was something more than from 19 to 22 days, a result which agrees well with the findings of the workers previously mentioned.

These and similar results, although they argue strongly in favor of the prolonged life of the red blood corpuscles after transfusion, have not been accepted as proof thereof, because they depend on the blood count uncontrolled by the volume of the blood. It is argued that the prolonged high blood count after transfusion may not represent an increased total number of corpuscles but an upset of long duration in the blood-volume-controlling factors, which results in a decrease in the fluid content of the blood and a consequent increased cell count.

Since the satisfactory determination of changes in the blood volume offers difficulties at present unsurmounted, it would seem that any method that could finally settle the question of whether or not the transfused blood corpuscles have any lasting existence in the blood stream must depend on some means of identifying the transfused corpuscle without in any way changing it by stains, etc. It seemed possible to do this in man by making use of the blood groups that are so well defined there. In man there are four blood groups, based on the ability of the serum of one group to agglutinate the corpuscles of another (Sanford). The corpuscles of Group I are agglutinated by the serum of all other groups but its serum has no agglutinating properties. The serums of Groups II and III mutually agglutinate each other's

corpuscles. Group IV serum agglutinates all other corpuscles, while Group IV corpuscles are not agglutinated by any serum. It follows then that a person in Group I may receive the blood of a person of any other group, and that the blood of a person in Group IV may be given to a person belonging in any of the other groups, since danger in transfusing blood of an unlike group arises only when there is agglutination of the incoming corpuscles, the transfused serum being too much diluted when mixed with the recipient's blood to produce any agglutination of his own corpuscles. Such transfusions, especially from the Group IV donor into the Group I, II, or III recipient, are fairly commonly done in the Mayo Clinic. I have found that when a blood whose corpuscles are agglutinable is treated with a serum capable of agglutinating them, the agglutination may, by using sufficient serum, be made practically complete. The count of the few corpuscles lying between the clumps (Fig. 1) is usually from 0.5 to 0.7 per cent of the total blood count, but it may vary in different bloods from 0.03 to 3.4 per cent. When, however, unagglutinable corpuscles are mixed with agglutinable corpuscles, either by transfusion or in the test-tube before the agglutinating serum is added, there is a very large number of free corpuscles present (Fig. 2) which appear in the proportion in which the two kinds of corpuscles are mixed. Since we have a means of separating the corpuscles of two groups that have been mixed by agglutinating the corpuscles of one group and leaving the corpuscles of the other unagglutinated, and since it is possible to transfuse a patient with a group other than his own, we may, by taking samples of his blood from time to time after transfusion and differentially agglutinating his own corpuscles, tell from the abnormal number of unagglutinated corpuscles present how long the transfused blood remains in the circulation.

### *Technique.*

Preliminary experiments were made with mixtures of bloods to determine the factors that it was necessary to control in order to obtain a technique which would give uniform results. Mixtures of corpuscles of known count were made; these were treated with the agglutinating serum, and after varying amounts of shaking, incubat-

ing, etc., a count was made of the unagglutinated corpuscles in a red blood cell counting chamber. In this preliminary procedure a series of mixtures of blood to serum 1:1, 1:5.5, 1:11, 1:22, and 1:55 was made. Although the 1:1 mixtures gave numerous unagglutinated corpuscles, 1:5.5 did not give a consistently greater percentage than any of the mixtures in which a greater proportion of serum was used; therefore, the 1 : 22 mixture, which was adopted because of its adaptability to use in the white cell counting pipette, should secure a maximum agglutination. Shaking during incubation probably frees some of the unagglutinable corpuscles that might otherwise be caught in the clumps of agglutinated corpuscles and thus increases the count of unagglutinated corpuscles in mixtures in which the unagglutinable corpuscles are present. Allowing the blood to stand in the ice box over night after incubation appears to give more uniform results. Slight differences in the length of time in the ice box caused no practical changes. Allowing the tubes to become warm after being in the ice box caused a decided increase in the count; thus it is advisable to keep each tube in the ice box until the count is to be made. Tubes may be thoroughly shaken to produce an even suspension before making a count without producing any increase in the unagglutinated corpuscles. 10 minutes shaking in a mechanical shaker with glass beads in the tube increases the count of the unagglutinated corpuscles only 1 to 2 per cent of the total blood count, and here heat rather than the shaking is probably the factor that causes the increase. If the unagglutinated corpuscles are so thick that the count cannot readily be made, it is possible to make a dilution with cold serum. It is not advisable to use normal salt solution as that increases the count. Partly as a result of this preliminary work and partly for the sake of uniformity, the following technique was adopted.

Blood is taken from the ear in a white cell counting pipette to the 0.5 mark, the pipette is then filled to the 11 mark with the agglutinating serum to which a 4.4 per cent citrate solution has been mixed in the proportion of 20 : 1, and the whole is expelled into a small test-tube and shaken, thus giving a 1 : 22 mixture of blood and citrated serum. This mixture is incubated at 37°C. for 40 minutes with thorough shaking every 10 minutes and is left in the ice box over night. The mixture is then thoroughly shaken and a drop of it is placed in a

red blood cell counting chamber and a count of the unagglutinated corpuscles is made. 160 squares in each of two chambers are counted, the average is taken, and the count multiplied by $\frac{1,100}{2}$ to give the number of unagglutinated corpuscles per cubic millimeter of blood. A tube containing the blood of a person who has not been transfused is always used to control the effectiveness of the serum, and serum from the same person is used for agglutinating throughout a set of experiments.

*Differential Agglutination in Vitro.*

*In vitro* experiments were done to establish whether or not the results obtained by agglutinating the agglutinable corpuscles in a mixture of unagglutinable and agglutinable corpuscles were quantitative, and to determine whether, in such a mixture, the number of unagglutinable corpuscles counted is equal to the number actually present or whether it is some function of that number. To show the accuracy of the technique under absolutely uniform conditions with the same blood and serum and simultaneous shaking and incubation, two tests were made of each of a series of mixtures with different pipettes and the results compared (Table I).

These figures show a degree of accordance between each of two distinct tests which leaves no doubt as to their quantitative character.

In order to compare the number of unagglutinated corpuscles with the known number of agglutinable corpuscles present, mixtures of a Group IV blood with a Group II blood were made by the drop method in the proportions of 1:30, 1:15, 1:10, etc.; the same capillary pipettes were used throughout. Samples of these mixtures, which had been thoroughly shaken, were diluted in the white cell counting pipette with the Group IV serum, after which the usual technique was followed. Two agglutinations were made from each mixture and the count of the unagglutinated cells was averaged after subtracting the number of unagglutinated corpuscles found in the pure Group II blood, which in this instance was rather high, being 119,900 per c.mm. The number thus obtained was compared with the number of the Group IV corpuscles computed to be present, and found by dividing

the count of the Group IV blood used by the fraction which it composed of the whole mixture. The results are shown in Table II.

Through a wide range of mixtures the counted number of unagglutinated corpuscles approximated closely the number of unagglutinable Group IV corpuscles computed to be present.

TABLE I.

| Proportion of Group IV corpuscles mixed with Group II corpuscles. | Red blood cell count of mixture. | Unagglutinated corpuscles in. | | Tube 1 minus Tube 2. | Percentage of difference based on total blood count. |
|---|---|---|---|---|---|
| | | Tube 1. | Tube 2. | | |
| | | | | | *per cent* |
| 1 : 30 | 4,880,000 | 235,400 | 209,000 | 26,400 | 0.58 |
| 1 : 25 | 4,870,000 | 249,700 | 228,800 | 10,900 | 0.22 |
| 1 : 20 | 4,840,000 | 335,500 | 325,600 | 9,900 | 0.20 |
| 1 : 15 | 4,800,000 | 474,200 | 464,200 | 6,600 | 0.14 |
| 1 : 10 | 4,710,000 | 578,600 | 566,500 | 12,100 | 0.26 |
| 1 : 5 | 4,500,000 | 894,300 | 887,700 | 6,600 | 0.15 |
| Average percentage of error..... | | | | | 0.25 |

TABLE II.

| Proportion of Group IV corpuscles mixed with Group II corpuscles. | Unagglutinated corpuscles per c. mm. minus 119,900. | Calculated No. of Group IV corpuscles per c. mm. |
|---|---|---|
| 1 : 30 | 127,370 | 137,000 |
| 1 : 15 | 228,570 | 267,000 |
| 1 : 10 | 391,600 | 388,100 |
| 1 : 6 | 572,773 | 610,000 |
| 1 : 4 | 803,559 | 854,000 |
| 1 : 3 | 1,035,320 | 1,067,500 |

*Evidence that the Unagglutinated Corpuscles Observed after Transfusion Are the Transfused Corpuscles.*

Before the abnormal increase of unagglutinated corpuscles found in the blood of Group I, II, or III patients transfused with Group IV blood could be assumed to be due to the presence of the transfused blood, it was considered necessary to run control experiments covering two points: (1) to see that this increase in count of unagglutinated

corpuscles does not appear when a patient with blood of an agglutinable group is transfused with blood of the same group, and, therefore, that the appearance of unagglutinated corpuscles is not some non-specific reaction incident to the introduction of foreign blood; (2) to see that the appearance of the unagglutinable corpuscles is not due to a reaction of the transfused Group IV serum on the recipient's native corpuscles.

It was also of interest to note, in as far as it was practicable with our insufficient knowledge of the blood volume, whether there was any correlation between the amount of blood transfused and the number of unagglutinated corpuscles which appear in the circulation following transfusion with unagglutinable blood. That the increase in unagglutinable corpuscles does not appear except when unagglutinable blood is transfused is shown in the following results.

Of four patients who were transfused with blood of a like group, one gave 91,300 per c.mm. unagglutinated corpuscles before transfusion and 42,900 per c.mm. after transfusion, when 200 cc. of blood were transfused. In the three other cases, when 500 cc. were transfused, 51,700, 52,800, and 8,800 per c.mm. of unagglutinated corpuscles respectively were counted. As the agglutination with no transfusion has varied within the limits of from 0.03 to 3.4 per cent of the blood count, these figures are well within normal limits.

On the assumption that the appearance of unagglutinated corpuscles might be due to the transfused serum, the following experiment was done. During a transfusion on a Group II patient which lasted less than 30 minutes and in which 500 cc. of citrated Group IV blood were transfused, samples of blood were taken from the ear, one after 300 cc. and one after 500 cc. of blood had been given. A control had been taken immediately before the transfusion. The results obtained by agglutinating with Group IV serum were: before transfusion 41,690 unagglutinated corpuscles per c.mm., after transfusion of 300 cc. 269,000 unagglutinated corpuscles per c.mm., and after transfusion of 500 cc. 478,390 unagglutinated corpuscles per c.mm. Samples of blood taken before transfusion, both citrated and uncitrated, in this case and in others gave no increase in the unagglutinated corpuscle count when mixed with Group IV serum in the proportion of 1 : 22 and incubated for 24 hours. It would seem that if *in vitro* Group IV

serum does not affect the agglutinability of Group II corpuscles during 24 hours at body temperature in over 100 times the concentration in which it appears in the recipient's body, the immediate increase in unagglutinable corpuscles obtained during transfusion is not due to a reaction of any constituents of the transfused serum on the recipient's native corpuscles.

Whether or not the unagglutinated corpuscles appear in numbers proportional to the amount of blood transfused is shown in seven patients, of whom six were transfused with Group IV blood and one was a Group I patient who was transfused with Group III blood. The count of the unagglutinated corpuscles was compared with a computed figure obtained from the amount of blood transfused, the body weight, and a factor common to the series. If the correction is omitted which would be necessary if differences exist in different persons in the proportion between blood and body weight, concerning which unfortunately we have no satisfactory data, it may be assumed that the number of unagglutinated corpuscles which appear should be directly as the amount of blood transfused and inversely as the body weight. Taking $x$ as the common unknown factor in Case A (Table III), in which the amount of blood transfused was 200 cc., the body weight 133.5, and the count of unagglutinated corpuscles 220,000, we have

$$220{,}000 = \frac{200}{133.5}\ x$$

Then by solving for $x$ whose value is 146,850 and substituting in a similar equation in Case B in which the amount of blood transfused was 500 cc. and the body weight 151 pounds, a computed figure will be obtained, which may be called $y$, for the number of Group IV corpuscles which should be present per c.mm. Thus

$$y = \frac{500}{151} \times 146{,}850$$

$$y = 486{,}258$$

If we continue this process through the series, computed figures are obtained which give a basis of comparison of the results obtained in different cases. A comparison of these computed figures, with the

counted number of corpuscles, is given in Table III. If due allowance is made for the inherent factor of error in the computed figures it will be seen that the degree of agreement between these two sets of figures indicates a quantitative relation between the amount of blood transfused and the number of unagglutinated corpuscles which appear in the circulation after transfusion. The weight at the time of transfusion was used in all instances except in Case B who could not be weighed.

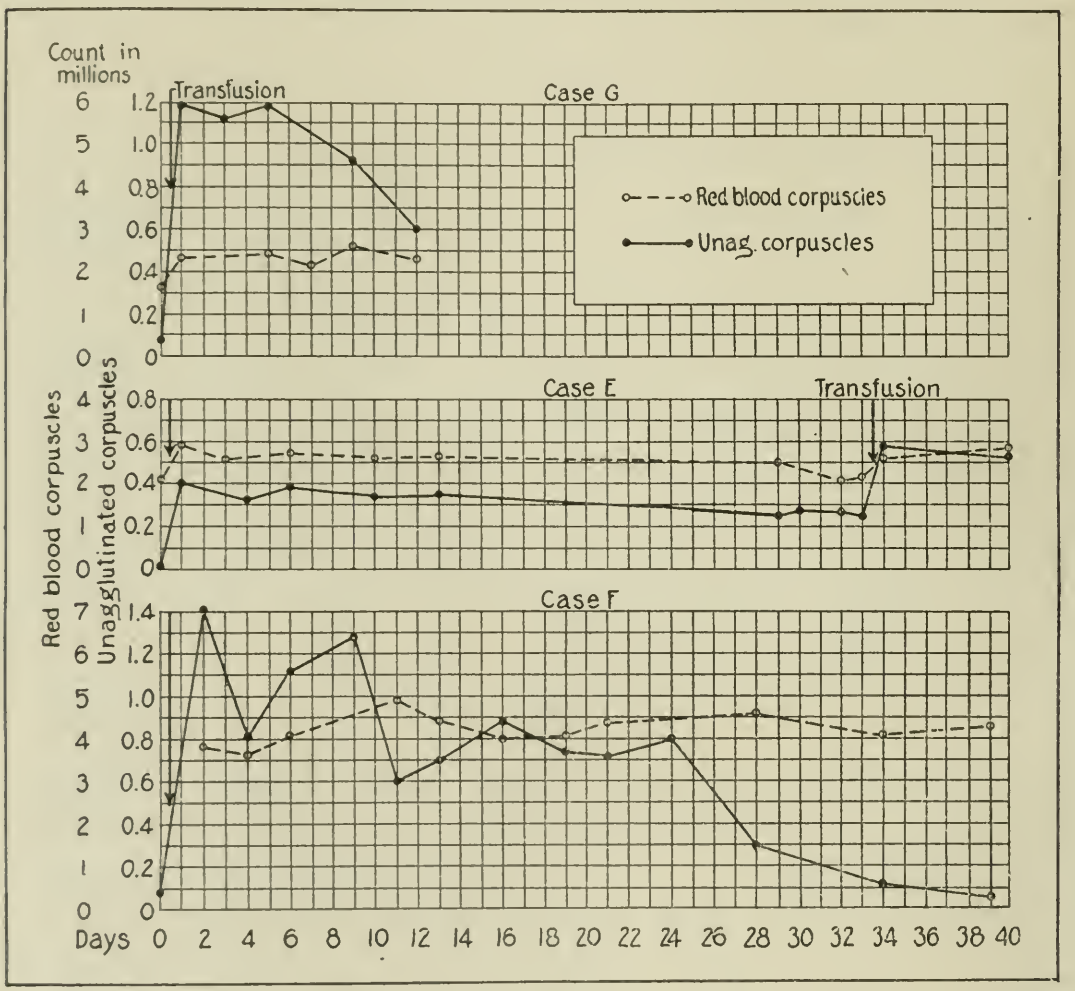
TABLE III.

| Case No. | Reason for transfusion. | Weight. | Amount of blood transfused. | Calculated No. of Group IV corpuscles per c. mm. | Counted unagglutinated corpuscles per c. mm. |
|---|---|---|---|---|---|
| | | *lbs.* | *cc.* | | |
| A | Preparatory to operation. | 133.5 | 200 | | 220,000 |
| B | Pernicious anemia. Transfused 20 days previously with 500 cc. of Group IV blood. | 151 (normal). | 500 | 486,258 | 551,000 |
| C | Hemorrhage following Kraske operation. | 96 | 500 | 764,843 | 698,000 |
| D | Pernicious anemia. Transfused 19 days previously with Group IV blood. The initial count of unagglutinated corpuscles was 10 per cent of the blood count. | 96 | 500 | 764,843 | 980,000<br>700,000 (minus initial count). |
| E | Anemia. | 135 | 500 | 543,888 | 409,200 |
| F | Hemorrhage following hysterectomy. | 138 | 1,200 | 1,276,956 | 1,412,400 |
| G | Anemia (infant). | 20.5 | 250 | 1,790,853 | 1,175,900 |

*Evidence of the Prolonged Existence of Transfused Blood Corpuscles.*

Application of this technique was undertaken for the purpose of determining whether or not transfused blood lives in the circulation, and specimens of blood of patients of an agglutinable group transfused with Group IV blood were taken before, and at short intervals after transfusion. These specimens were examined for the persistence after transfusion of abnormal numbers of unagglutinated cor-

puscles. The protocols of Cases G, E, and F give the counts of unagglutinated corpuscles and a simultaneous study of the total blood count (Text-fig. 1). These figures represent the average of two pipettes. The unagglutinated corpuscles of the normal untransfused Group II individual who was used as a control varied through the experiments from 0.5 to 0.7 per cent of the total blood count.




TEXT-FIG. 1. The rate of change in the number of transfused corpuscles per cubic millimeter and the accompanying changes in the total blood count.

*Case G.*—Fat infant, age 17 months; weight 20.5 pounds. Belongs to Group II; had a low blood count. A diagnosis of secondary anemia had been made Sept. 20, 1918. The red blood cells were 1,660,000; the unagglutinated corpuscles were 54,400 per c.mm. A transfusion was done of 250 cc. of citrated Group IV blood.

Sept. 21. Red blood corpuscles 2,375,000; unagglutinated corpuscles 1,175,000 per c.mm.
" 23. " " 1,107,700 " "
" 25. Red blood corpuscles 2,380,000; " " 1,192,400 " "
" 27. " " " 2,260,000.
" 29. " " " 2,570,000; unagglutinated corpuscles 933,900 per c.mm.
Oct. 2. " " " 2,280,000; " " 602,250 " "
" 4. Sent to the hospital with an attack of influenza.
" 7. Died.

*Case E.*—A Group II individual, age 32 years. Had a secondary type of anemia, possibly from hemorrhage due to piles. The patient's normal weight was 160 pounds, the present weight was 135 pounds. Oct. 1, 1918. The red blood cells were 2,100,000; the unagglutinated cells were 1,100 per c.mm.

A transfusion was done with 500 cc. of Group IV blood.

Oct. 12. Red blood corpuscles 2,820,000; unagglutinated corpuscles 409,200 per c.mm.
" 14. " " " 2,550,000.
" 15. Unagglutinated corpuscles 318,000 per c.mm.
" 17. Red blood corpuscles 2,705,000; " " 390,700 " "
Mild attack of influenza.
" 21. Red blood corpuscles 2,600,000; unagglutinated corpuscles 333,700 per c.mm.
" 24. " " " 2,660,000; " " 367,400 " "
" 25. Went home for 2 weeks.
Nov. 9. Red blood corpuscles 2,510,000; unagglutinated corpuscles 251,020 per c.mm.
" 10. " " 260,700 " "
" 12. Red blood corpuscles 2,105,000; " " 258,720 " "
" 13. " " " 2,185,000; " " 246,070 " "
A transfusion was done of 500 cc. of Group IV blood.
" 14. Red blood corpuscles 2,625,000; unagglutinated corpuscles 585,200 per c.mm.
" 20. " " " 2,760,000; " " 517,000 " "

*Case F.*—Age 35 years; normal weight 140 pounds; present weight 138. Operated on for fibroid of the uterus. Sept. 21, 1918. The unagglutinated cells were 88,000 per c.mm.; the blood count could not be obtained. 700 cc. of Group IV blood were given because of severe hemorrhage; the hemorrhage continued and an additional 500 cc. of Group IV were given.

Sept. 23. Red blood corpuscles 3,800,000; unagglutinated corpuscles 1,412,400 per c.mm.
" 25. " " " 3,720,000; " " 807,400 " "
" 27. " " " 4,090,000; " " 1,130,800 " "
" 30. " " 1,293,800 " "
Oct. 2. Red blood corpuscles 4,920,000; " " 590,700 " "
" 4. " " " 4,405,000; " " 704,000 " "
" 7. " " " 4,000,000; " " 870,100 " "
" 10. " " " 4,060,000; " " 737,000 " "
" 12. " " " 4,250,000; " " 702,900 " "
" 15. " " 796,400 " "
" 19. " " " 4,550,000; " " 299,200 " "
" 25. " " " 4,109,000; " " 118,800 " "
" 30. " " " 4,302,000; " " 51,700 " "

The greater irregularities in the count of unagglutinated corpuscles in this protocol are due to the fact that the patient's blood often clotted the Group IV serum with which it was mixed. This tendency was overcome later by increasing the strength of the citrate solution from 2 per cent to 4.4 per cent.

It will be noted in these cases that in each instance before transfusion there was an initial count of unagglutinated corpuscles which came within the limits of the counts found in other untransfused persons, while after transfusion there was a very great increase in unagglutinated corpuscles. In Case F, a patient who had had a severe hemorrhage and in whom the amount of blood transfused was great, this increase amounted to over 30 per cent of the total blood count; in Case G in whom the initial blood count was extremely low and the amount of blood given in proportion to the body weight was also great, it amounted to nearly 50 per cent of the patient's blood count. The increase was maintained for a long time with a surprisingly slow fall. In Case G who died 5 days after the last count was taken, only half the number of unagglutinated corpuscles which appeared after transfusion had disappeared in 12 days; in Case E whose condition remained on a level notwithstanding a slight attack of grippe, there was no marked decrease in the original number of unagglutinable corpuscles after 13 days, and in 33 days there was only a 37 per cent fall; in Case F, a comparatively normal patient who made an excellent recovery from operation, there was a marked decrease in unagglutinated corpuscles between the 25th and 28th days, and the agglutinability of the patient's blood may be considered to have approximately reached normal by the 30th day. In all cases the unagglutinated corpuscles, as long as they were present in considerable numbers, appeared to be in good condition.

I have shown that this differential agglutination reaction is a constant and quantitative one and that the unagglutinated corpuscles are present in numbers equal to the unagglutinable corpuscles admixed, so that unless something takes place in the body that does not take place in the test-tube, the counts of unagglutinated corpuscles after transfusion may be assumed to be a quantitative indicator of the number of transfused corpuscles present. As the unagglutinated corpuscles appear after transfusion in numbers proportionate to the amount of unagglutinable blood transfused, as they do not appear

when agglutinable blood is transfused, and, moreover, as they are not caused by any effect of the Group IV serum on the recipient's corpuscles, it seems safe to assume that these unagglutinated corpuscles are the transfused corpuscles and that their number is a quantitative indicator of the amount of transfused blood present in the recipient's circulation. It must follow then that the prolonged appearance of unagglutinated corpuscles in the blood stream of these transfused patients can only mean that the transfused corpuscles existed for a long time in the circulation, and it seems that they not only existed but functioned.

In considering these results, the question of to what extent they apply to the more usual condition when the patient is transfused with blood of his own group, immediately arises. Since the peculiarities which are brought out in blood grouping are probably peculiarities which will also be found in all the cells of the body, we may be able to turn to other tissues for light on this point. In this connection Masson's work in skin grafting is of interest.

"In all patients requiring skin grafting who were under my care during the past year, the blood of the donor, as well as that of the recipient, has been tested for agglutination. The results have been very interesting and instructive, and I feel sure will add a great deal to the popularity of the use of the isograft. . . . . I have tested the principle with the three varieties of grafts, and am satisfied that blood grouping is just as important for good results in skin grafting as it is necessary in transfusion, and that it is governed by the same principles. While the results are not positive, nevertheless, I have never had a skin take which was removed from a donor whose red blood corpuscles were agglutinated by the serum of the patient. The results in all other cases have been very satisfactory, almost, if not entirely, equal to autodermic grafting."

It would seem probable, if further investigation bears out the above results with skin grafts, that the results obtained in studying the length of life of transfused Group IV corpuscles in a recipient of another group hold for the life of the corpuscles of any group transfused into the recipient of a like group, and that it is even probable that these results hold for the length of life of the native corpuscle.

Since my results show that there is no immediate breaking down of transfused cells which could cause a stimulation of the bone marrow, and since it is more probable, as suggested by Lindeman, that when

there is a stimulation of the bone marrow following transfusion it is due to improved metabolic conditions of the bone marrow cells, brought about by the larger number of corpuscles in the blood, it would seem, on the surface, that the logical procedure in transfusion would be to push the transfusions until a normal blood count was attained, making the time interval between transfusions only long enough to allow the fluid content to establish itself. This would be indicated by the rise in the blood count which takes place a day or two after transfusion.

## CONCLUSIONS.

1. It is possible in mixtures of corpuscles of different groups to separate the corpuscles practically quantitatively by treating with a serum that agglutinates the corpuscles of one kind, leaving the others unagglutinated.

2. After a recipient has been transfused with blood of a group other than his own, specimens of his blood treated with a serum that will agglutinate his own corpuscles but not the transfused corpuscles show unagglutinated corpuscles in large numbers.

3. These unagglutinated corpuscles which appear in the recipient's blood after such a transfusion are the transfused corpuscles and their count is a quantitative indicator of the amount of transfused blood still in the recipient's circulation.

4. The life of the transfused corpuscle is long; it has been found to extend for 30 days and more. The beneficial results of transfusion are without doubt not due primarily to a stimulating effect on the bone marrow, but, it is reasonable to assume, to the functioning of the transfused blood corpuscles.

## BIBLIOGRAPHY.

Archibald, A., The transfusion of blood in the treatment of pernicious anemia, *St. Paul Med. J.*, 1917, xix, 43.

Brown-Sequard, quoted by Hunter.

Crile, G. W., Hemorrhage and transfusion, New York and London, 1909, 560.

Hunter, W., Duration of life of red corpuscles, as ascertainable by transfusion, *Proc. Roy. Soc. Edinburgh*, 1884–86, xiii, 849.

Kimpton, A. R., Transfusion. Experiences in over two hundred cases, *Boston Med. and Surg. J.*, 1918, clxxviii, 351.

Lindeman, E., A new method for estimating total blood volume in anemias. Preliminary report, *J. Am. Med. Assn.*, 1918, lxx, 1209.

Lindeman, E., The total blood volume in pernicious anemia, *J. Am. Med. Assn.*, 1918, lxx, 1292.

Marfels and Moleschott, quoted by Hunter.

Masson, J. C., Skin grafting, *J. Am. Med. Assn.*, 1918, lxx, 1581.

von Ott, quoted by Hunter.

Primrose, A., The value of the transfusion of blood in the treatment of the wounded in war, *Ann. Surg.*, 1918, xlviii, 118.

Quinck, quoted by Hunter.

Robertson, L. B., and Watson, C. G., Further observations on the results of blood transfusion in war surgery, *Ann. Surg.*, 1918, lxvii, 1.

Sanford, A. H., Selection of the donor for transfusion, *Journal-Lancet*, 1917, xxxvii, 698.

Ward-Muller, quoted by Hunter.

## EXPLANATION OF PLATE 14.

FIG. 1. Microphotograph of the agglutinated blood of a person not transfused.

FIG. 2. Microphotograph showing the clumps of agglutinated native corpuscles and the unagglutinated transfused corpuscles of blood of an individual 16 days after transfusion.

# EXPERIMENTS ON THE PRODUCTION OF SPECIFIC ANTISERA FOR INFECTIONS OF UNKNOWN CAUSE.

## I. Type Experiments with Known Antigens—a Bacterial Hemotoxin (Megatheriolysin), the Pneumococcus, and Poliomyelitic Virus.

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There are two generally recognized prerequisites to any attempt at the production in animals of an antiserum to combat an infectious disease. These are, needless to state, the isolation and the successful cultivation *in vitro* of the disease's causative agent. The difficulties here encountered in the case of some common and important maladies have absolutely held up all advances toward specific treatment.

The possibility of immunizing animals by direct injections of infected human tissues is one that must have occurred to many minds; and some attempts to employ the method were made prior to the general recognition that tissue itself acts to engender antibodies highly injurious to the species from which it is derived. With that recognition the method was given up, and it has not been revived. Yet there are noteworthy instances, such as the Pasteur treatment for rabies, which prove that tissue may contain an infectious agent in sufficient quantity to act as a practical antigen. And living tissues are superior in one respect to all other culture media, since organisms flourishing in them possess those pathogenic characters, loss of which in the test-tube sometimes limits, or indeed prevents, the development of antibodies in animals injected with cultures.

It has seemed to us possible that an antiserum produced by the injection of infected tissues might be rendered fit for employment in the species from which the tissues are derived by submitting it to a process of selective absorption, or exhaustion, with suspensions of

normal tissues. Such treated serum would, supposedly, be deprived of injurious tissue antibodies while retaining those directed against the infectious agent. A number of difficulties at once suggest themselves. It is well known that the absorption of antibodies is to a remarkable degree selective; and yet may not the repeated absorption of a polyvalent serum with tissue cells weaken notably the general content in antibodies? Will not toxic elements develop as a result of the incubation *in vitro* of mixtures of serum and tissue? And even should this not occur, is it really possible by the method of absorption to rid a serum of its anti-tissue potency so far as to render it harmless *in vivo*? May not the success of the absorptions be dependent on the employment of tissues derived from the same organ or organs used in the immunization? And finally, will not the method after all be more tedious than practical?

Some of these questions can be answered out of present knowledge. The recent work of many investigators on anaphylatoxins has shown that toxic elements are indeed engendered in incubated mixtures of tissue and its antibody, but only when complement is present.[1] In our selective absorptions inactivated serum could and should be used. The amount of tissue required to exhaust a serum of even high anti-tissue titer need not be great, since, as has been repeatedly shown in the typical case of red cells, many times the minimum hemolytic unit of amboceptor or agglutinin may be taken from a serum by a single unit of antigen. Furthermore, in the case of most sera, one can hope to use red corpuscles instead of tissues of the precise sort employed in the immunization. For much previous work has clearly demonstrated that only in certain special instances are specific cytotoxins the result of tissue injections.[2] Usually hemolysins, hemagglutinins, and serum precipitins alone develop.

In the work to be described our aim has been to determine the fundamental point, whether sera obtained by the immunization of animals with infected tissue of another species can, by the method of absorption, be rendered available for therapeutic use in the last mentioned species. No attempt has been made to devise practical

[1] See, for example, Friedberger, E., *Z. Immunitätsforsch.*, 1910, iv, 636.

[2] Pearce, R. M., *J. Med. Research*, 1904, xii, 1.

methods of absorption, to determine the least amount of tissue that will exhaust a serum, etc. For the purpose of type experiments sera have been selected containing different kinds of antibodies directed in all except one case—that of the chicken tumor—against pathogenic agents already isolated and well studied.

*Selective Absorption Applied to an Antitoxic Serum.*

The simplest case, that of an antitoxic serum, was first taken up. In view of the possible importance of "anaphylatoxins" engendered in the incubated mixture of serum and tissue as above mentioned, it seemed best to work with the animals most susceptible to such poisons; namely, guinea pigs. Furthermore, it was desirable to employ, if possible, a toxin of which the neutralization with antitoxin could be observed *in vitro* as well as *in vivo*. Both conditions are met by the hemolysin produced by *Bacillus megatherium* and first studied by Todd.[3] Todd demonstrated that the lysin is a true toxin, against which an antitoxin can be readily produced in guinea pigs, rabbits, and goats. His work has been fully confirmed.[4] *Bacillus megatherium* produces its toxin only when grown *in vitro* under special conditions, and although the toxin will kill guinea pigs, the organism itself is practically non-pathogenic. For this reason infected tissues could not be obtained for the immunizations required by our plan. Normal tissues mixed with the toxin might perhaps have been used, but the latter by itself, when injected locally, causes a violent inflammatory reaction. It was decided, on this account, to inject the toxin and the tissue used for immunization at separate sites and at different times.

*Characters of the Megatheriolysin.*—Three strains of *B. megatherium* were obtained from the American Museum of Natural History through the kindness of Professor Winslow. Only one produced any noteworthy hemolysin when cultivated in the special bouillon recommended by Todd.[5] This produced a lysin of such strength that 1 cc. of the Berkefeld filtrate of a 7 day culture, when in-

[3] Todd, C., *Lancet*, 1901, ii, 1663; *Tr. Path. Soc. London*, 1902, liii, 196.

[4] Dreyer, G., and Blake, J., *Lancet*, 1904, ii, 408. Craw, J. A., *Proc. Roy. Soc. London, Series B*, 1905, lxxvi, 179. Vincent, H., *Compt. rend. Soc. biol.*, 1909, lxvii, 195.

jected intravenously into a 400 gm. guinea pig, regularly caused intense hemolysis and hemoglobinuria, with death in a few hours at most. Intraperitoneally 2 cc. produced death quite as rapidly from diffuse petechial hemorrhages, first into the mucosa of the small intestine, and thence into the lumen of the intestine, which became distended with fluid blood. The uterus, large intestine, and stomach sometimes showed petechiæ, usually scattering. Despite the striking local lesions there was no evidence of intravascular hemolysis and never any hemoglobinuria, even when death occurred slowly. When given subcutaneously the lysin caused a wide area of necrosis.

The strain of *B. megatherium* furnishing the lysin was cultivated in quantity in Todd's medium, and at the end of 7 days the culture fluid was centrifuged and passed through a Berkefeld filter, after which the filtrate was tested for sterility, tubed, the tubes sealed with paraffin, and stored in the dark at 2–3°C. Under these conditions the lysin was found to retain practically all its activity for months, a great advantage, since portions of the same filtrate could be used both for the immunization of animals and for tests of the antisera that they yielded.

## *Immunization of Animals.*

The lysin is most injurious to guinea pigs, yet large amounts are ill tolerated by rabbits or goats. The normal serum of both these animals has some slight neutralizing activity for the lysin, as shown by the ability to prevent hemolysis of guinea pig corpuscles. Attempts were made at first to immunize rabbits. A number of these animals were given six intraperitoneal injections at 6 day intervals of defibrinated guinea pig blood plus suspensions of ground liver and spleen, followed 2 days later by intraperitoneal injections of 1 cc. of the megatheriolysin. The animals were bled 7 days after the last lysin injection. They stood the immunization badly; all lost weight, and several died. Nevertheless, as will be seen, the survivors elaborated a well marked antitoxin.

A goat was immunized by separate subcutaneous injections of tissue and lysin according to the method just outlined. The finely ground liver, spleen, and kidney of guinea pigs, and the defibrinated blood were mixed and used. The amount of megatheriolysin given was gradually increased from 0.5 to 10 cc., diluted always with salt solution. This caused boggy swellings which were slow to subside. 7 days after the last of the six lysin injections the goat was bled, and the serum was tubed without the addition of preservatives and left in the ice box. The rabbit serum was similarly treated.

## *Method of Exhaustion with Red Cells.*

The serum was inactivated at 56°C. for ½ hour just prior to use. Guinea pig red cells taken into citrate were thrice washed in ¼ per cent gelatin Locke's solution under aseptic conditions. In ordinary salt solutions guinea pig corpuscles

are prone to break down when washed; but the addition of a little gelatin to the washing fluid will prevent this.[5] After the last washing the cells were packed in graduated tubes, their bulk was noted, all possible fluid pipetted off, and the serum to be exhausted poured on, the tube corked, inverted, and gently shaken to suspend the cells. The suspension was now warmed to 38°C. in the water bath, incubated, centrifuged at high speed, and the serum transferred to a fresh lot of cells. The period of incubation varied with the degree of agglutination of the cells. When they fell out promptly into a solid mass such as could not be broken up without hemolysis, incubation for more than a few minutes was manifestly useless. But often after one or two absorptions the cells tended to remain in suspension, and the incubation period was lengthened to an hour. Finally, when the suspended cells showed no trace of agglutination, incubation was continued for as long as 2 hours in some instances.

No preservative was added to the mixture of serum and cells, but great care was taken to assure its sterility. The special corks for centrifuge tubes, elsewhere described,[5] were an aid in this connection. After the last absorption cultures were regularly taken. These showed an entire absence of infection, which may perhaps be attributed as much to the frequent centrifugation at high speed to which the serum was subjected as to our technique.

With repeated absorption there was a slight unavoidable loss of serum, and a slight dilution of it also occurred, owing to the remnant of gelatin Locke's solution introduced with the cells. In testing the relative potency of the unexhausted and exhausted serum usually no correction was made for this dilution of the latter.

## *Specimen Experiments—Rabbit Serum.*

### *Experiment 1.*

For this and all the subsequent tests the sera were inactivated at 56°C. for ½ hour. Whenever the period of incubation of the *in vitro* mixture is not specifically mentioned it was for 2 hours at 38°C. Readings were made after the tubes had stood over night in the ice box.

*Hemolytic Activity of the Megatheriolysin.*—This was determined as follows: 0.25 cc. of megatheriolysin in graded dilutions + 0.25 cc. of 0.9 per cent sodium chloride + 0.25 cc. of a 5 per cent suspension of washed guinea pig red cells.

| Lysin strength* | $\frac{1}{5}$ | $\frac{1}{10}$ | $\frac{1}{20}$ | $\frac{1}{40}$ | $\frac{1}{80}$ | $\frac{1}{160}$ | $\frac{1}{320}$ |
|---|---|---|---|---|---|---|---|
| Hemolysis | C. | C. | C. | C. | +++ | Ft. Tr. | 0 |

* The lysin strength is expressed in terms of the undiluted material.

*Antilytic Titer of Normal Serum.*—The antilytic titer was tested of the inactivated sera of four normal rabbits, as contrasted with that of an animal repeatedly injected with megatheriolysin and guinea pig tissues. Mixtures were made of

[5] Rous, P., and Turner, J. R., *J. Exp. Med.*, 1916, xxiii, 219.

the sera in graded dilutions with a fixed amount of megatheriolysin, guinea pig red cells were added, and incubation was done. The amount of lysin in each tube was more than eight times that necessary under ordinary conditions for complete hemolysis of the corpuscles (*vide supra*). Subsidiary tests which need not here be given in detail showed that rabbit serum exercised its whole neutralizing effect on the lysin practically at once when mixed with it at room temperature. Consequently no interval was allowed to elapse before the red corpuscles were added.

0.25 cc. of rabbit serum in graded dilutions + 0.25 cc. of $\frac{1}{5}$ strength megatheriolysin + 0.25 cc. of 5 per cent guinea pig red cells.

| Serum. | | Serum dilution. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 |
| Normal. | A | ++ | +++ | ++++ | Alm. C. | C.(?) | C. | C. | C. |
| | B | + | + | ++ | +++ | ++++ | Alm. C. | " | " |
| | C | + | + | ++ | ++ | +++ | " " | " | " |
| | D | + | ++ | ++ | ++++ | ++++ | " " | " | " |
| Immune | | 0 | 0 | 0 | Tr. | +++ | " " | " | " |

It is evident from this experiment that the normal rabbit sera possessed some power to prevent destruction of corpuscles by the megatheriolysin. But the immune serum conferred at least eight times as much protection, as shown by comparing its effect, when diluted, with that of the concentrated normal sera. The failure of the immune serum to protect to the same proportional degree in the higher dilutions is attributable to the presence in megatheriolysin of several lytic components[3] against all of which doubtless the antiserum had not the same relative activity.

*Anti-Guinea Pig Titer of the Immune Serum.*—This was well marked as a result of the repeated injection of the immunized rabbit with guinea pig tissue. A precipitin was present effective against dilutions of guinea pig serum up to and including 1: 256 when an equal bulk of the concentrated immune serum was mixed with it. Tests for hemolysis and agglutination were made as follows:

0.25 cc. of serum dilution + 0.25 cc. of 1 in 10 guinea pig complement + 0.25 cc. of 5 per cent guinea pig red cells.

| | Serum dilution. | | | | | | | | | | | Complement + red cells + 0.25 cc. of salt solution. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | 1/1,024 | |
| Hemolysis | 0 | + | +++ | ++ | ± | ± | Tr. | Tr. | Ft. Tr. | 0 | 0 | 0 |
| Agglutination | C. | C. | C. | C. | C. | C. | ++++ | ± | Tr. | 0 | 0 | 0 |

The low hemolytic titer was doubtless due in part to serum precipitation which took place in the mixtures, for complement is absorbed during such precipitation, as is well known. But it is also explained by the close biological relationship of rabbit and guinea pig which renders difficult the production of antibodies in the one against the other. The fact should also be recalled that specific hemolysins as a rule act but weakly when complemented with serum from the species furnishing the test cells. Yet it was deemed best to use such complement in our tests, since it would be the only one present during *in vivo* experiments.

*Selective Absorption of the Anti-Guinea Pig Elements.*—3 cc. each of the four normal sera and the immune serum above mentioned, all inactivated, were mixed respectively with 1.5 cc. of sedimented guinea pig red cells, incubated for 1 hour, and centrifuged, and the serum was transferred to a fresh portion of red cells. In the case of Normal Sera C and D only 0.5 and 0.65 cc. respectively of red cells were employed in the second absorption, while for the others 1.5 cc. were used as before. Incubation again was for 1 hour. In the first mixture of immune serum and cells a moderate agglutination was to be seen. None of the other mixtures ever showed the least trace of clumping.

Tests were made after the second absorption to determine how completely hemolysin and agglutinin had been removed from the immune serum. No trace of either was found. In view of these results tests to show whether the normal rabbit sera had been completely exhausted were deemed unnecessary, since such sera when untreated are almost devoid of antibodies for guinea pig red cells.

*Antilytic Titer of the Treated Immune Serum.*—0.25 cc. of immune serum in graded dilutions + 0.25 cc. of $\frac{1}{5}$ megatheriolysin + 0.25 cc. of 5 per cent guinea pig red cells.

| Serum. | Serum dilution. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 |
| Untreated | 0 | 0 | 0 | Tr. | +++ | Alm.C. | C. | C. |
| Exhausted | 0 | 0 | 0 | +++ | Alm. C. | C. | " | " |

The exhaustion with red cells had but little diminished the antimegatheriolytic titer of the serum.

*In Vivo Tests of the Neutralization of Megatheriolysin with Exhausted Serum.*—A number of mixtures containing 1 cc. of undiluted megatheriolysin with 0.75 cc. of serum or salt solution were incubated 2 hours, and 1.5 cc. of each were injected directly into the ear vein of a guinea pig. The method of injection has been described elsewhere.[6] The long period of incubation was unnecessary, since neutralization of the lysin is, as has been stated, practically instantaneous at room temperature.

[6] Rous, P., *J. Exp. Med.*, 1918, xxvii, 459.

| Guinea Pig No. | Weight. | Mixture used for injection. | Result. |
|---|---|---|---|
| | *gm.* | | |
| 1 | 350 | Exhausted immune serum + lysin. | Remained well. |
| 2 | 350 | Untreated " " + " | Died immediately after injection. |
| 3 | 375 | Exhausted normal serum (No. 1) + lysin. | Died 6 hrs. later; intense hemolysis; hemoglobinuria. |
| 4 | 350 | Exhausted normal serum (No. 2) + lysin. | Died 10 hrs. later; intense hemolysis; hemoglobinuria. |
| 5 | 375 | Exhausted normal serum (No. 3) + lysin.* | Died 40 hrs. later; intense hemolysis; hemoglobinuria. |
| 6 | 375 | Exhausted normal serum (No. 4) + lysin. | Died 10 hrs. later; intense hemolysis; hemoglobinuria. |
| 7 | 350 | Salt solution + lysin. | Died 13 min. later; intense hemolysis; at autopsy characteristic lesions. |
| 8 | 350 | " " + " | Died 12 hrs. later; intense hemolysis; hemoglobinuria. |

* Part of injection material lost.

All the animals that succumbed, except No. 2, showed the lesions already described as characteristic of the megatheriolysin. In this case death was practically immediate, and there were no gross lesions except an almost complete intravascular hemagglutination, caused, of course, by the untreated immune serum, and without doubt the cause of death.

## *Experiment 2.*

Another less comprehensive experiment with immune rabbit serum will be briefly quoted, since in addition to showing the antitoxic power of the exhausted serum it affords an interesting contrast between the lesions caused by the megatheriolysin, as such, and mixtures of the lysin with unexhausted serum.

The serum of an immunized rabbit was incubated as already described with two successive batches of washed guinea pig red cells. *In vitro* tests showed that the hemolysin and agglutinin were thus removed, whereas the antilysin was practically as strong as ever. The following mixtures were now made: (*a*) 2 cc. of megatheriolysin + 1.25 cc. of serum or salt solution; (*b*) 4 cc. of megatheriolysin + 2.35 cc. of serum or salt solution. After 2 hours incubation at 38°C. the whole of each mixture was injected into the peritoneal cavity of a guinea pig.

| Guinea Pig No. | Weight. | Mixture (*a*). Lysin + | Result. |
|---|---|---|---|
| | *gm.* | | |
| 9 | 480 | Exhausted immune serum. | Remained well; no anemia. |
| 10 | 480 | Untreated " " | Died after 48 hrs.; extreme anemia; hemoglobinuria. |
| 11 | 480 | Salt solution. | Died after 4½ hrs.; lesions characteristic of the megatheriolysin. |
| | | Mixture (*b*). Lysin + | |
| 12 | 500 | Exhausted immune serum. | Remained well; no anemia. |
| 13 | 500 | Untreated " " | Died after 48 hrs.; progressive anemia; hemoglobinuria. |
| 14 | 520 | Salt solution. | Died after 1½ hrs.; lesions characteristic of the megatheriolysin. |

The hemoglobin percentage in the blood of the surviving animals was followed for some days.

At autopsy the animals receiving megatheriolysin + salt solution presented the findings already described as characteristic after intraperitoneal injections. The small intestines were distended with blood from many fine hemorrhages into the mucosa. Other hemorrhages were present in the walls of the large intestine and stomach. The blood remaining in the vessels was unclotted, greatly concentrated, but unhemolyzed and unagglutinated. There was never any hemoglobinuria. The lesions from mixtures of the lysin with unexhausted serum were entirely different, being those characteristic of a serum hemolysin. A severe progressive anemia developed, accompanied by hemoglobinuria, and the blood specimens showed marked agglutination and many shadows. At autopsy there was no trace of intestinal hemorrhages such as result from the megatheriolysin. The spleen was greatly enlarged and crowded with phagocytes filled with red cells. There were also scattered ecchymoses on the pleuræ, diaphragm, and parietal peritoneum—a lesion never observed as the result of the megatheriolysin but commonly produced by a serum hemolysin. The conclusion is unavoidable that the animals had been saved from the action of the megatheriolysin only to succumb to that of the serum hemolysin.

### *Specimen Experiment—Goat Serum.*

### *Experiment 3.*

A freshly prepared lysin was employed for the work. Its titer diminished so little in the course of the 6 weeks during which a goat was repeatedly injected with it that tests made of the neutralizing activity of the serum of the animal before and after the immunization can be directly compared.

*Hemolytic Activity of the Lysin.*—0.2 cc. of megatheriolysin in graded dilutions + 0.2 cc. of 5 per cent guinea pig red cells.

| Hemolysis. | Lysin strength. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1/5 | 1/10 | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 |
| At first test..... | C. | C. | C. | C. | C. | Alm. C. | +++ | Tr. |
| 6 wks. later..... | " | " | " | " | Alm. C. | " " | + | " |

*Antilytic Titer of the Goat Serum before and after Immunization.*—These tests were made at the same time as those of the megatheriolysin just quoted. 0.25 cc. of goat serum in graded dilution + 0.25 cc. of $\frac{1}{8}$ strength megatheriolysin + 0.25 cc. of 5 per cent guinea pig red blood cells.

| Serum. | Serum dilution. | | | | | | | | | Whole strength serum + red cells + salt solution. |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | |
| Prior to immunization..... | +++ | +++ | ++++ | Alm. C. | Alm. C. | Alm. C. | C. | C. | C. | 0 |
| After immunization (6 wks. later)..... | 0 | 0 | 0 | ± | C. | C. | " | " | " | |
| Serum of a normal goat (control)..... | Alm. C. | C. (?) | C. | C. | " | " | " | " | " | |

The serum of the normal control was obtained and tested at the same time as the immune serum.

Plainly the immunization with megatheriolysin had increased the antilytic titer of the goat serum.

*Anti-Guinea Pig Elements and Their Selective Absorption.*—Preliminary tests showed that the serum of the immunized goat contained powerful antibodies for guinea pig red cells, as would naturally follow from the repeated injection of the animal with tissues of this species. An attempt was made to absorb the antibodies from a portion of the serum, and the titer of the exhausted specimen was then compared with that of an untreated portion. The normal control serum was not submitted to absorption because it was found to be harmless to guinea pigs when given in the doses required by our experiments.

For the purposes of exhaustion 17 cc. of the immune serum were incubated for 1 hour with 5 cc. of washed guinea pig red cells, the mixture was centrifuged, and

the serum transferred to more red cells and incubated again. This was done five successive times. In the first mixtures the red cells were only moderately agglutinated, but in the later ones clumping became much more marked owing doubtless to the absorption of proagglutinoids, which at first had hindered agglutination.

| Mixture. | Hemagglutination. |
|---|---|
| 17 cc. of serum + 5 cc. of red cells incubated 1 hr. and | Moderate. |
| serum transferred to 4.25 cc. of red cells, incubated 1 hr. and | " |
| " " " 7.6 " " " " " 1 " " | Strong. |
| " " " 7.3 " " " " " 1 " " | Almost massive. |
| " " " 5.2 " " " " " 1 " | Moderate. |

Only 13.5 cc. of serum were finally recovered. The diminution in volume was due to a retention of the fluid amidst the agglutinated red cells. The latter, when clumped by the serum, occupied a greater space than when simply sedimented in salt solution.

A comparison was now made of the hemolytic and agglutinative titer of the exhausted and unexhausted immune serum, and of the normal control serum. 0.2 cc. of inactivated goat serum in graded dilutions + 0.2 cc. of 1 in 10 guinea pig complement + 0.2 cc. of 5 per cent guinea pig red cells were used.

| | Serum dilution. | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | 1/1,024 | 1/2,048 | 1/4,096 |
| Serum. | Hemolysis. | | | | | | | | | | | | |
| Untreated normal..... | +− | Tr. | Tr. | Ft. Tr. | 0 (?) | 0 | 0 | 0 | 0 | 0 | | | |
| Untreated immune..... | Ft. Tr. | ++− | ++ | + | Tr. | Ft. Tr. | Ft. Tr. | − | 0 | 0 | | | |
| Exhausted immune..... | No hemolysis. | | | | | | | | | | | | |
| Serum. | Agglutination. | | | | | | | | | | | | |
| Untreated normal..... | No agglutination. | | | | | | | | | | | | |
| Untreated immune..... | +++ | + | ++ | C. | C. | C. | C. | C. | C. | ++ | + | Tr. | 0 |
| Exhausted immune..... | +++ | +++ | +++ | ++ | + | ± | 0 | 0 | 0 | 0 | | | |

Agglutination was read in the same mixtures as hemolysis. With rabbit complement the untreated immune serum was found to be far more hemolytic than with guinea pig complement as here shown. Precipitation was observed in the hemolytic mixtures containing immune serum in dilutions up to 1: 64, and to this is attributable the Neisser-Wechsberg phenomenon observed in the hemolytic tests of the untreated specimen.

*Antilytic Titer of the Treated Immune Serum.*—0.25 cc. of serum in graded dilutions + 0.25 cc. of $\frac{1}{8}$ megatheriolysin + 0.25 cc. of 5 per cent guinea pig red cells.

| Serum. | Serum dilution. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 |
| Untreated immune..... | 0 | 0 | 0 | ± | C. | C. | C. | C. | C. |
| Exhausted " ..... | 0 | 0 | 0 | ++ | " | " | " | " | " |
| Untreated normal..... | Alm. C. | C. (?) | C. | C. | " | " | " | " | " |

The repeated absorption of the immune serum with large amounts of red cells (29.35 cc. of the latter in all, as against only 17 cc. of serum) was found scarcely to affect the antimegatheriolytic titer, which remained more than eight times that of the normal serum.

## *In Vivo Tests of the Neutralization.*

(*a*) *Intraperitoneal Injections.*—Mixtures of 2 cc. of megatheriolysin (or salt solution) with 2.5 cc. of serum (or salt solution) were incubated 1 hour at 38°C and injected into a number of guinea pigs of 400 gm. weight.

| Guinea Pig No. | Mixture injected. | Result. |
|---|---|---|
| 15 | Normal serum + salt solution. | Remained well; no anemia. |
| 16 | 0.9 per cent salt solution + lysin. | Died after 1¼ hrs. with characteristic lesions. |
| 17 | Normal serum + lysin. | " " 12 " " " " |
| 18 | Unabsorbed immune serum + lysin. | " " 53 " " progressive anemia and hemoglobinuria. |
| 19 | Absorbed immune serum + lysin. | Remained perfectly well save for a slight anemia soon repaired. |

The order of injection was as follows: Nos. 15, 17, 19, 18, 16.

The findings at autopsy were similar to those of Experiment 2 with rabbit serum, as already described. All the animals that died, save No. 18, showed

lesions characteristic of the action of the megatheriolysin. In the guinea pig mentioned the lesions were of a different sort, being such as are caused by a specific hemolysin; and no trace of injury from the megatheriolysin was to be seen.

(*b*) *Intravenous Injections.*—Mixtures of 1 cc. of lysin (or salt solution) with 0.8 cc. of serum (or salt solution) were incubated 1 hour at 38°C., and 1.5 cc. of each mixture were injected into the ear vein of a guinea pig.

| Guinea Pig No. | Mixture injected. | Result. |
|---|---|---|
| 20 | Normal serum + salt solution. | Remained well; no anemia. |
| 21 | " " + lysin. | Died after 42 hrs. with progressive anemia and hemoglobinuria. |
| 22 | Salt solution + " | Died after 47 hrs. with progressive anemia and hemoglobinuria. |
| 23 | Untreated immune serum + lysin. | Died after 8 hrs.; prostrated at once; hemoglobinuria. |
| 24 | Exhausted immune serum + lysin. | Remained well save for a moderate anemia. |

Guinea Pig 20 weighed 425 gm., the others 450 gm.
The order of injection was as follows: Nos. 24, 23, 21, 20, 22.

## *Comment.*

It is clear that by the method of selective absorption an antitoxic serum strong in tissue antibodies can be deprived of the latter to such extent as to be converted from a highly injurious agent into one capable of saving life. Indeed such a serum submitted to absorption five successive times, with a total bulk of corpuscles almost twice its own volume, was found to retain practically all its titer in antitoxin (Experiment 3).

A few of the guinea pigs receiving mixtures of exhausted serum and megatheriolysin were temporarily prostrated, lying on the side, and twitching, but they soon got to their feet and showed no permanent injury. The prostration occurred as frequently after the injection of normal serum as of that from animals immunized against guinea pig tissues. It is not possible to say whether the symptoms were due to "anaphylatoxin," or resulted from struggle under durance in connection with the rapid intravenous injection of a relatively large amount of foreign fluid. No such symptoms were ever observed after intraperitoneal inoculations.

The animals saved by the action of exhausted immune serum gave no evidence of injury to liver, spleen, or kidney, such as might perhaps have been expected in view of the fact that suspensions of these organs mixed with defibrinated blood were employed in the immunization. However, the only urine test made was for hemoglobin. Some of the guinea pigs receiving exhausted serum developed a slight or moderate anemia which was slow to appear and was most marked 4 or 5 days after the injection. Special experiments which need not here be cited in detail showed convincingly that the blood injury was due, not to unneutralized megatheriolysin, but solely to insufficient exhaustion of the serum with red cells, as proved by the persistence in it of hemagglutinins. When the absorptions were continued until all hemagglutinins had been removed, the serum was harmless to the blood. For example, it was found that the serum of Experiment 3 after seven absorptions no longer possessed hemagglutinins or produced anemia, whereas after only five absorptions it had both these characters as our protocol shows. The point is an important one, suggesting that the complete absence of hemagglutinins may be taken as the index to when exhaustion of a serum is complete. This indicator has been adopted, in much of our later work, and properly, as the results show. Hemagglutinins are far stronger than hemolysins in most sera resulting from prolonged immunization with animal tissues, and persist long after the latter have been removed by absorption.[7]

*Selective Absorption Applied to an Antipneumococcus Serum.*

The results of the work with an antitoxic serum were so encouraging that experiments were begun with sera of other types. It was highly desirable that they should be developed through the actual employment of infected tissues as antigen. The pneumococcus was selected for some of the tests, and attempts were made to immunize dogs against the organism by means of injections with the tissues of rabbits dying of pneumococcus septicemia. The difficulties encoun-

[7] An exception is to be noted in the case of anti-chicken sera from the goose and rabbit. These regularly contain hemolysins in fair quantity but only weak hemagglutinins.

tered illustrate strikingly the differences which may exist in the pathogenicity of a microorganism growing *in vivo* and *in vitro*.

Dogs possess a considerable resistance to the pneumococcus compared with some other species, as is well known; and they will often withstand the intravenous injection of several cubic centimeters of a bouillon culture fatal in minute quantity to mice. Intraperitoneal inoculations are even better borne. Nevertheless the immunization of dogs against the pneumococcus by intraperitoneal injections of blood or other tissues from rabbits moribund with pneumococcus septicemia proved well-nigh impossible, because of the high virulence of the antigen and our inability to standardize that derived from different rabbits. Small amounts of the infected tissue caused the dogs to die with a pneumococcus septicemia. Not infrequently they withstood a number of injections, only to succumb to one which was, quantitatively speaking, inconsiderable. In order to avoid this the infected rabbit tissue was heated *in vitro* at temperatures between 40° and 50°C. prior to injection, and the number of living organisms was thus reduced, as cultures showed, from millions to but a few per cubic centimeter. Still the injections often resulted fatally. Separate intraperitoneal inoculations of pneumococcus cultures and of normal tissues gave better results, but pneumococcus peritonitis so often ensued that at length separate subcutaneous inoculations were decided upon. These were carried out over a period of several months and a serum was finally obtained of sufficient antipneumococcus and anti-tissue titer to be suitable for experiments on selective absorption. We can confirm the observation of Nuttall[8] and Doerr and Moldovan[9] that antibodies of high titer are with difficulty elicited in the dog, as a response to immunization.

The plan of the experiments required yet a further alteration. Rabbits were found to vary so markedly in their resistance to the pneumococcus that very many would have been required had they been used as test animals in protection experiments with the antipneumococcus serum. It was decided, on this account, to exhaust the dog serum with rabbit red cells, in the manner that had proved successful with antimegatheriolytic serum and guinea pig cells, but to

[8] Nuttall, G. H. F., Blood immunity and blood relationship, Cambridge, 1904.

[9] Doerr, R., and Moldovan, J., *Z. Immunitätsforsch., Orig.*, 1910, vii, 223.

carry out protection tests with mice instead of rabbits. Mice were found to tolerate well the intraperitoneal injection of normal dog serum in the amount necessary for the work.

*Immunization of Animals.*—Nine dogs weighing from 8¼ to 13 kilos were injected intravenously with amounts varying from 0.5 to 1.25 cc. of the mixed citrated blood of three rabbits moribund with pneumococcus septicemia. The organism was of Type I (Neufeld strain), and the infected blood was preserved in the frozen condition for 4 days prior to use. Five of the dogs died of pneumococcus septicemia within a few days after the injection. The surviving four animals received injections of antigen at intervals of 7 days for more than 2 months. At first citrated septicemic blood was given intraperitoneally. This was badly tolerated, so recourse was had to inoculations with normal tissue and bouillon cultures of the pneumococcus, given at separate subcutaneous sites and on different days. The normal tissue consisted of suspensions of finely ground rabbit liver, spleen, and kidney mixed with defibrinated blood. Kidney tissue in special was employed, with the object of obtaining a serum that would be nephrotoxic. None of the dog sera acquired a demonstrable nephrotoxin, however, though hemolysins and hemagglutinins soon developed, and also weak agglutinins for the pneumococcus. The two most highly immunized dogs (Dogs A and B), as judged by these features, were bled for serum 9 days after the last pneumococcus injection.

*Exhaustion of the Serum.*—The method of selective absorption was that already described in connection with antimegatheriolytic serum. Rabbit red cells, thrice washed, were packed by rapid centrifugation, all possible fluid was removed, and the cells were mixed and incubated with the serum under test. The latter was in this way exhausted by contact with several successive portions of cells.

*Experiment.*—The sera of two immunized dogs and three normal controls were inactivated at 56°C. for ½ hour, and portions of all were submitted to an exactly similar exhaustion with red corpuscles. The incubation period was 1 hour with each successive batch of cells.

| Mixture. | Hemagglutination. | | |
|---|---|---|---|
| | Immune sera. | | Normal sera. |
| | A | B | |
| 26 cc. of serum + 3⅓ cc. of red cells, incubated 1 hr. and | Massive. | Heavy. | |
| serum transferred to 3⅓ cc. of red cells; incubated 1 hr. | Moderate. | Moderate. | |
| " " " 4 " " " " " 1 " | Slight. | Slight. | 0 |
| " " " 4 " " " " " 1 " | Tr. | Ft. Tr. | |

Cultures taken after the last absorption showed all the sera to be sterile.

*Anti-Rabbit Titer of the Immune Sera. Hemolysis.*—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 1 in 10 guinea pig complement + 0.2 cc. of 5 per cent red cells.

| Serum. | | Serum dilution. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | 1/1,024 |
| Dog A | Untreated.... | C. | C. | +++ | ++ | ++− | + | + | Tr. | Ft. Tr. | 0 | 0 |
| | Exhausted.... | | | No hemolysis. | | | | | | | | |
| Dog B | Untreated.... | C. (?) | Alm. C. | Alm. C. | ++++ | ++ | +− | +− | Tr. | Ft. Tr. | 0 | 0 |
| | Exhausted.... | | | No hemolysis. | | | | | | | | |

*Hemagglutination.*—The mixtures were the same as those for hemolysis save that 0.2 cc. of salt solution was substituted for guinea pig complement.

| Serum. | | Serum dilution. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | 1/1,024 |
| Dog A | Untreated.. | C. | C. | C. | C. | +++ | +++ | ++ | Tr. | Tr. | 0 | 0 |
| | Exhausted.. | ++++ | +++ | ++ | Tr. (?) | 0 | 0 | | | | | |
| Dog B | Untreated.. | C. | C. | Alm. C. | ++++ | ++++ | ++ | Tr. | 0 | 0 | 0 | 0 |
| | Exhausted.. | ++ | Tr. | 0 | 0 | | | | | | | |

Although some hemagglutination was noted in the mixtures with exhausted serum after they had stood over night, no trace of this was observable when they were first taken from the incubator. Many hemagglutinins, as Landsteiner and Reich[10] first showed, act most strongly at low temperature.

Because of these findings, which showed that the exhaustion of the immune sera with rabbit cells had been practically complete, no similar tests were made of the exhausted normal sera. For the anti-rabbit titer of these sera, when unexhausted, was, relatively speaking, slight.

*Pneumococcus Agglutinins.*—These were present in the immune sera but were weak, being effective on macroscopic test only in dilutions up to 1 in 16 of each serum. They were found to be unaffected by exhaustion of the sera. The details of the tests need not be given.

*In Vivo Tests of the Protective Power of the Exhausted Antipneumococcus Serum.*—With the change in plan that determined the use of mice instead of

[10] Landsteiner, K., and Reich, M., *Centr. Bakteriol., 1te Abt., Orig.*, 1905, xxxix. 83.

rabbits for the protection experiments, it became unnecessary to make *in vivo* tests as to whether the sera exhausted with red cells had been deprived of their toxicity for the species furnishing the cells; that is to say, for rabbits. The results with exhausted anti-guinea pig serum of far higher original titer, which have already been described, were deemed sufficient on the point, especially since tests showed that the unexhausted dog serum contained not the least nephrotoxin for the rabbit, despite the repeated use of the renal tissue of rabbits as an antigen. The method adopted for the protection experiments with mice was that now familiar from the work of Avery, Chickering, Cole, and Dochez.[11] An 18 hour culture in pneumococcus broth of the Neufeld strain of pneumococcus was used in a series of tenfold dilutions with this broth. Of each culture dilution 0.5 cc. was drawn into a Record syringe, then 0.5 cc. of the serum under test was taken up, and the whole was at once injected into the peritoneal cavity of a 20 gm. mouse. The control sera were handled after the immune sera, so that the former had the advantage of any attenuation in the pneumococcus suspensions which might have occurred during the period of the injections. Last of all, some mice were given a set of control mixtures containing 0.5 cc. of broth instead of a serum.

The animals that died were autopsied promptly and films taken of the peritoneal exudate and heart's blood. These in every case showed the pneumococcus in pure culture. No mouse was put down as surviving until 4 days after injection.

| Serum. | | Amount of culture. | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.000001 cc. | 0.00001 cc. | 0.0001 cc. | 0.001 cc. | 0.01 cc. | 0.1 cc. |
| | | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* | *hrs.* |
| Broth controls | | D.* in 26 | D. in 28 | D. in 32 | D. in 20 | D. in 16 | |
| Immune A | Unexhausted | Lived. | Lived. | " " 42 | " " 26 | " " 23 | D. in 17 |
| | Exhausted | " | " | Lived. | " " 89 | Lived.† | " " 11 |
| Immune B | Unexhausted | " | " | " | Lived. | D. in 17 | " " 14 |
| | Exhausted | " | " | " | D. in 32 | " " 49 | Lived. |
| Normal a | Unexhausted | D. in 30 | D. in 30 | D. in 23 | " " 17 | " " 17 | D. in 7½ |
| | Exhausted | " " 31 | " " 21 | " " 20 | " " 16 | " " 11 | " " 9 |
| Normal b | Unexhausted | " " 27 | " " 30 | " " 25 | " " 22 | " " 12 | " " 9½ |
| | Exhausted | " " 31 | " " 50 | " " 22 | " " 11 | " " 9 | " " 9 |
| Normal c | Unexhausted | " " 30 | " " 42 | " " 19 | " " 14 | " " 14 | " " 7½ |
| | Exhausted | " " 40 | " " 28 | " " 28 | " " 22 | " " 12 | " " 10 |

* D. indicates died.

† Slight escape of injected fluid beneath skin.

[11] Avery, O. T., Chickering, H. T., Cole, R., and Dochez, A. R., Acute lobar pneumonia. Prevention and serum treatment, Monograph of The Rockefeller Institute for Medical Research, No. 7, New York, 1917.

*Comment.*

The experiment shows that the immune dog sera protected mice against about 100 times the amount of pneumococci that was fatal when normal dog serum was employed. The exhaustion of the immune sera with four successive portions of rabbit red cells did not diminish in the least its protective character. The question may be asked, why was such an oblique method used to demonstrate the availability of the exhausted serum? Instead of immunizing dogs with rabbit tissue, exhausting the dog serum with rabbit red cells, and testing protection on mice, might not these latter animals have been employed throughout, to the elimination of rabbits? This was not practicable for several reasons. It would have been difficult to obtain enough sterile normal tissue from mice for the production of a strong anti-mouse serum, and, granting that such a serum could eventually have been elicited, the problem would have arisen of obtaining sufficient mouse red cells for its exhaustion. Undoubtedly the spleens of mice dying of pneumococcus infection would have furnished a powerful antigen, as concerns this organism; but so little of the splenic tissue could have been employed in the immunization, owing to the virulence of the pneumococci therein contained, that it is doubtful whether the serum resulting would have been strongly anti-mouse. And a serum strong in anti-tissue elements was desirable for our type experiments.

*Selective Absorption Applied to a Serum Conferring Protection against Poliomyelitis.*

As enlarging the general scope of the work, a test was made of whether exhaustion with red cells would deprive the serum of a monkey recovered from poliomyelitis of its protective power against this disease.[12]

The serum of an immune monkey might conceivably be used in the treatment of human beings after exhaustion of its anti-human elements. For this reason the selective absorption was carried out with human cells, though in the ultimate test of protection monkeys were of

[12] The experiment was rendered possible through the cooperation of Dr. Amoss.

necessity employed. The choice of cells was a poor one, because the anti-human elements in monkey serum are extremely weak[13] and are not readily enhanced by tissue injections. The test of the persistence of antipoliomyelitic elements in serum submitted to selective absorption with human cells is in consequence not a drastic one.

*Experiment.*—A *Macacus rhesus* monkey recently recovered from poliomyelitis, and with severe residual paralyses, was given intravenously on 3 successive days portions of a mixture of defibrinated human blood and an extract in salt solution of human placenta ground with sand. 10 days after the last injection the animal was bled for serum. This on test showed no hemolysin for human red cells and only weak agglutinins. It was exhausted as follows, according to the usual technique.

| Mixture. | Hemagglutination. |
|---|---|
| 2.25 cc. of serum + 0.5 cc. of human red cells, incubated 5 min.; | Massive. |
| and serum transferred to 0.25 cc. of human red cells, incubated 45 min.; | Moderate. |
| and serum transferred to 0.25 cc. of human red cells, incubated 45 min. | Faint. |

The exhaustion was nearly complete, as shown by tests in which one part of serum in graded dilutions + one part of 5 per cent human red blood cells were mixed in Wright's tubes and examined microscopically after 15 minutes at room temperature.

*Agglutination.*

| Serum. | Serum dilution. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 |
| Untreated | C. | — | — | + | +— | Ft. Tr. | 0 |
| Exhausted | + | Tr. | | | | | |

The test of protective power was carried out by Dr. Amoss, who mixed 2 cc. of the exhausted serum with 0.2 cc. of freshly prepared poliomyelitic virus, and injected the whole, after 2 hours incubation, into the cerebrum of a normal *rhesus* monkey. The animal was one of a considerable number receiving an equal amount of the same virus mixed with various sera, so the experiment was well controlled. Eight monkeys were given mixtures of 0.2 cc. of virus + 2 cc. of

[13] Marshall, H. T., *J. Exp. Med.*, 1901–05, vi, 347.

normal human or monkey serum, and all came down with poliomyelitis after from 5 to 7 days and died. The animal receiving exhausted immune serum mixed with virus remained entirely free from the disease.

The indication from this one experiment is clear, that the principle neutralizing the virus of poliomyelitis persists in immune serum exhausted with red cells. Owing to the difficulty of obtaining immune monkeys the work has not been repeated.

## SUMMARY.

Attempts to produce antisera in animals to combat specific infections are usually deferred until the cause of the infection has been isolated and grown in pure culture to furnish antigen. It has seemed to us that the fulfillment of these conditions might in some cases be rendered unnecessary through the use of infected tissue itself as an antigen, combined with selective absorption of the antiserum to rid it of elements injurious to the species furnishing the tissue. In order to test this possibility type experiments have been carried out with immune sera effective against known antigens of three different sorts:

1. Sera resulting from the injection of rabbits and a goat with normal guinea pig tissues and a bacterial hemotoxin, the megatheriolysin described by Todd, which hemolyzes guinea pig cells. The sera possessed strong antitoxins for the megatheriolysin but were fatal to guinea pigs. By the method of selective absorption they were rendered innocuous to these animals and were successfully used to protect them from lethal doses of the megatheriolysin.

2. Anti-rabbit dog sera containing antibodies protective against pneumococcus infection. Such sera, subjected to repeated absorption with rabbit red cells, proved capable of protecting mice from pneumococcus infection in exactly the same degree as the unexhausted serum; that is to say, they protected against 100 times the dose of pneumococci that was fatal with normal dog serum.

3. The serum of a monkey recovered from poliomyelitis and repeatedly injected with human red cells and extract of placental tissue. This serum, after selective absorption with human red cells, protected a monkey against an intracerebral dose of poliomyelitic virus

fatal to eight other monkeys given it with normal monkey or human serum.

The results in these instances, purposely chosen for their simplicity, would seem to indicate for the absorption method some usefulness in the study of immunity to infections of unknown cause. In Part II of our paper the method is applied to one such infection; namely, a sarcoma of the fowl engendered by a filterable agent. A general discussion will be found in connection with this portion of the work.

# EXPERIMENTS ON THE PRODUCTION OF SPECIFIC ANTISERA FOR INFECTIONS OF UNKNOWN CAUSE.

## II. The Production of a Serum Effective against the Agent Causing a Chicken Sarcoma.

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The work detailed in Part I of this paper has served to demonstrate the theoretical usefulness of exhausted sera to combat infections, but it presents no instance of a serum actually resulting from the direct immunization of animals by injections of infected tissue. Such an instance is highly desirable. It has been furnished through experiments with a transplantable chicken sarcoma,[1] known in our laboratory as Chicken Tumor I, which has a filterable agent as its cause. The exact nature of the filterable agent is unknown, but its general characters would seem to place it with the microorganisms.[2] The tumor is a typical sarcoma, highly malignant, and as a rule rapidly fatal to fowls developing it after an implantation with neoplastic tissue or inoculation with the Berkefeld filtrate of a tumor suspension. Some individuals are primarily insusceptible, and in some the growth develops slowly, and eventually retrogresses. The latter fail ordinarily to develop a sarcoma when reinoculated. Repeated unsuccessful attempts have been made to demonstrate antibodies in the blood of fowls in which a growth has retrogressed, and to render others immune to the tumor by injections with heated or dried neoplastic tissue.[3] The tumor cannot be transmitted to geese, ducks, pigeons, or mammals; but attempts to develop an antiserum by the immunization of such animals have been blocked through failure to obtain the tumor-

[1] Rous, P., *J. Exp. Med.*, 1910, xii, 696; *J. Am. Med. Assn.*, 1911, lvi, 198.
[2] Rous, P., and Murphy, Jas. B., *J. Am. Med. Assn.*, 1912, lviii, 1938.
[3] Rous, P., and Murphy, Jas. B., *J. Exp. Med.*, 1914, xx, 419.

producing agent in culture. The employment in these alien species of the neoplastic tissue itself as an antigen, or a filtrate from such tissue, elicits, of course, anti-chicken elements in the immunized individual.[4] The method of specific absorption to obtain an antiserum here finds a direct application.

*Immunization of Animals.*—The blood of fowls carrying the chicken tumor often contains during the last few days of life the causative agent of the disease; and in the sarcomatous tissue the agent is regularly present in large quantity. Both blood and tissue could therefore be used in the immunization, which was desirable in order to insure the production of a strong anti-chicken serum. Chickens moribund with the growth were bled to death under aseptic conditions, the blood was citrated, and the tumor tissue itself was ground with sand and suspended in Locke's solution just prior to injection. As the causative agent of the growth will withstand repeated freezing and thawing and retains its activity for a long period at low temperature, the material often was kept in the frozen state for days or weeks prior to use.

The first attempts to obtain an antiserum were made with rabbits. A number of these animals were injected intravenously on 3 successive days with a tumor extract in salt solution, and thereafter intraperitoneally every 6 days with citrated chicken blood and a suspension of tumor tissue. But though the serum soon acquired a high content of chicken hemolysins and hemagglutinins it had not the least neutralizing effect on the tumor-causing agent present in Berkefeld filtrates of suspensions of the sarcoma tissue. For this reason work with rabbits was at length discontinued.

Implanted bits of the chicken sarcoma perish at once in mammals, whereas in ducks and pigeons they grow for some days before retrogressing and may form quite large nodules. It seemed from this fact not improbable that birds would prove more favorable than rabbits as producers of tumor antibodies, owing to what might be considered as a partial susceptibility on their part to the neoplastic disease. For Flexner and his associates[5] have shown that in the case of poliomyelitis an immune serum is obtained only in species susceptible to the infection. Geese were used, therefore, in the further attempts to obtain an antiserum Their immunization was carried out as follows:

Goose A received three intravenous injections on successive days of mixed tumor suspension and citrated blood from fowls moribund of the growth, followed thereafter every 6 or 7 days by intraperitoneal injections of the same material. Goose B was given the same sort of material, but only into the peritoneal cavity. From time to time both birds were bled from a wing vein and the sera compared

[4] Bailey encountered this difficulty in experiments on complement fixation with the serum of pigeons inoculated with the growth (Bailey, C. H., *Med. Rec.*, 1915, lxxxviii, 403).

[5] Personal communication from Dr. Flexner.

in neutralizing properties with those of three normal geese. During a long period of immunization no differences could be noted, but on the contrary a similarity in the five sera so entire as to indicate that the blood of one normal goose is just like that of any other in its effect, or rather lack of effect, on the tumor-producing agent. At length, as will be seen from the experiments now to be cited, an immune principle became demonstrable in the serum of the injected birds.

*Experiment 1.*—The immunized geese, A and B, were bled for serum 84 and 66 days respectively after immunization was begun, and 9 days after the last injection of tumor material. Goose A had received three intravenous and ten intraperitoneal injections, while Goose B had but nine injections, all intraperitoneal. Two normal geese (a and b) were bled at the same time and to an equal amount; namely, 75 cc. The sera were inactivated as usual, and all were submitted to absorption with similar portions of washed chicken red cells, as follows:

| Mixture. | Hemagglutination. | |
|---|---|---|
| | Immune sera. | Normal sera. |
| 25 cc. of goose serum + 5.2 cc. of chicken red blood cells incubated 1 hr. and | + | 0 |
| serum transferred to 4 cc. of chicken red blood cells, incubated 1 hr. | Tr. | 0 |

Cultures taken after the second absorption proved sterile. Preliminary tests showed that the untreated immune serum failed to hemolyze chicken cells when chicken serum was used as complement, whereas these were rapidly destroyed in the presence of guinea pig complement. Consequently the latter was used in the titrations that follow.

*Anti-Chicken Titer of the Sera. Hemolysis.*—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 1 in 10 guinea pig complement + 0.2 cc. of 5 per cent guinea pig red cells. Incubation and ice box was for 2 hrs. at 38°C. and readings were made after the tubes had stood in the ice box over night.

| Serum. | | Serum dilution. | | | | | | | | Complement + red cells + 0.2 cc. of salt solution. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | |
| Untreated immune. | A.. | C. | C. | C. | Alm. C. | Alm. C. | +++ | Tr. | Ft. Tr. | 0 |
| | B.. | ++++ | +++ | ++ | + | +− | Tr. | Ft. Tr. | 0 | |
| Untreated normal. | a... | Tr. | Ft. Tr. | 0 | 0 | 0 | 0 | | | |
| | b... | " | " " | Ft. Tr. | 0 | 0 | 0 | | | |
| Exhausted sera... | | No hemolysis by any. | | | | | | | | |

The selective absorption had completely deprived the immune sera of their relatively strong hemolysin.

*Hemagglutination.*—This was read in mixtures similar to the foregoing but containing chicken serum (1 in 10) as complement. None of the tubes showed any hemolysis with this complement, but those containing undiluted immune goose serum exhibited a slight hemagglutination. None of the exhausted sera agglutinated chicken cells in the least.

*Precipitation.*—The normal sera contained no precipitin, but a weak one was present in the immune sera. It was active against dilutions of chicken serum up to, and including, 1 in 40.

*In Vivo Tests of Neutralization.*—The exhausted sera only were used in neutralization tests. For this purpose mixtures were made of the sera with a Berkefeld filtrate containing the tumor-producing agent, and these after incubation were injected into fowls. In some early experiments mixtures of the filtrate with isotonic saline or Locke's solution were employed as controls, but it was found that they soon lost their tumor-producing activity when incubated, whereas this was retained in mixtures with normal goose serum, either untreated or exhausted. Consequently in the present experiment, as in others to be detailed, the mixtures with normal sera constitute the controls.

The tumor filtrate was prepared by grinding fresh neoplastic tissue with sand, making a thin suspension in Locke's solution, shaking, centrifuging, and passing the clear fluid through one or another of several Berkefeld filters (N). Several filters were used to ensure an active filtrate, since the tumor-producing agent is held back by many of the finer Berkefeld candles, and all the filtrates were united. Now two mixtures were made with the sera: (1) 15 cc. of each exhausted serum + 7 cc. of filtrate; (2) 7 cc. of each exhausted serum + 2 cc. of filtrate. These were incubated for 2 hours at 38°C. They remained water-clear. 1 cc. of a suspension of sterile diatomaceous earth was added to each, and portions of all were injected into each of a number of chickens. The mixtures with immune sera were injected first so that any possible advantage as regards attenuation of the virus during incubation, or neutralization of it, might lie with the mixtures containing the normal serum. Diatomaceous earth was added because, through the tissue injury it causes, the production of tumors by a filtrate is rendered much more certain.[6]

The ten chickens inoculated received 3 cc. of each mixture, into the pectoral muscles and the muscles of the upper wings respectively. Usually the tumor grows fastest and becomes largest in the pectoral muscles, and for this reason the injection site for the mixtures was varied from bird to bird; but in the experiment now under consideration no favoring influence of the pectoral situation

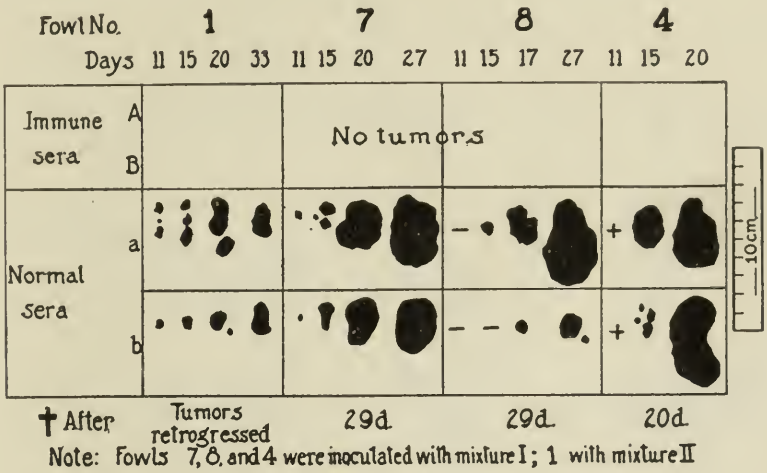
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[6] Rous, P., Murphy, Jas. B., and Tytler, W. H., *J. Am. Med. Assn.*, 1912, lviii, 1751.

was to be seen. The growths did not attain a very large size before death ensued from metastases.

Clear-cut findings were obtained, as Text-fig. 1 shows. Only four of the ten fowls developed tumors. In them growths failed to appear where the mixtures of immune sera and filtrate had been injected, whereas at the control sites large ones developed.

*Experiment 2.*—The same general plan was followed as in the preceding experiment, but the immunized geese had now received two additional intraperitoneal injections. Bleeding for serum was done 121 and 103 days respectively from the time immunization of the birds was started, and 7 days after the last




TEXT-FIG. 1. The tumors in four fowls receiving intramuscular injections of mixtures of tumor filtrate with immune and normal goose sera respectively.

injection. The sera of three normal geese, a, b, and c, were used in control. Selective absorption was carried out as usual.

30 cc. of goose serum + 5.8 cc. of chicken red cells incubated 1 hr. and
serum transferred to 2.9 " " " " " " 1 " "
" " " 2.8 " " " " " " 1 "

Cultures taken after the last absorption proved sterile.

*Anti-Chicken Titer of the Sera. Hemolysis.*—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 1 in 10 guinea pig complement + 0.2 cc. of 5 per cent chicken red cells.

| Serum. | | Serum dilution. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 |
| Untreated immune. | A..... | C. | C. | C. | Alm. C. | ++ | + | Tr. | 0(?) | 0 |
| | B..... | Alm. C. | Alm. C. | +++ | ++ | + | Ft. Tr. | 0 | 0 | 0 |
| Untreated normal. | a..... | No hemolysis. | | | | | | | | |
| | b..... | +− | 0 | 0 | 0 | 0 | | | | |
| | c..... | +++ | ++ | + | 0 | | | | | |
| Exhausted sera..... | | No hemolysis by any. | | | | | | | | |

*Hemagglutination.*—0.2 cc. of inactivated serum in graded dilutions + 0.2 cc. of 5 per cent chicken red cells + 0.2 cc. of salt solution.

| Serum. | | Serum dilution. | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1/2 | 1/4 | 1/8 | 1/16 |
| Untreated immune. | A..... | + | + | + | Tr. | 0 |
| | B..... | ++ | Tr. | 0 | 0 | 0 |

With the exhausted normal and immune sera, as well as the untreated normal sera, no agglutination was obtained.

*Precipitation.*—There was no precipitin in the normal sera, but one was present in that from both immune geese. It was effective in mixtures of equal parts of the undiluted goose serum with dilutions of chicken serum up to and including 1 in 40 for Goose A and 1 in 20 for Goose B. The titer was little if at all diminished by the absorption with red cells.

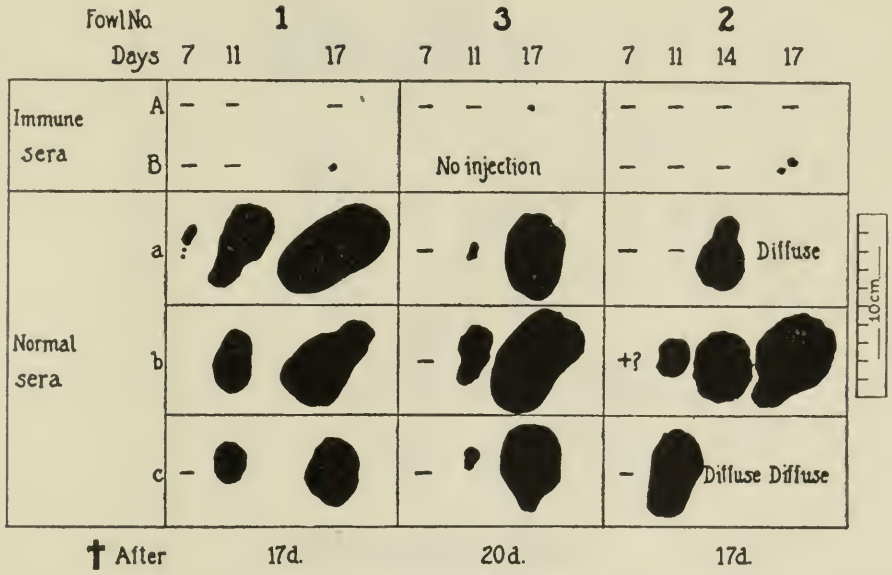
*In Vivo Tests of Neutralization.*—A Berkefeld filtrate of a tumor extract was prepared by the method already described, and three mixtures were made of it with the exhausted sera, both normal and immune.

Proportion X: 7.5 cc. of serum + 2 cc. of filtrate.
Proportion Y: 12 cc. of serum + 6 cc. of filtrate.
Proportion Z: 7 cc. of serum + 7 cc. of filtrate.

Incubation was for 2 hours at 37°C. No precipitation or clouding occurred. A suspension of diatomaceous earth was now added to each mixture in the amount of one-tenth its volume, and the injection of fowls was forthwith begun. Fifteen fowls were used, and all save four received 3 cc. of each mixture, the site of injection being varied. The four fowls mentioned were not given the mixture con-

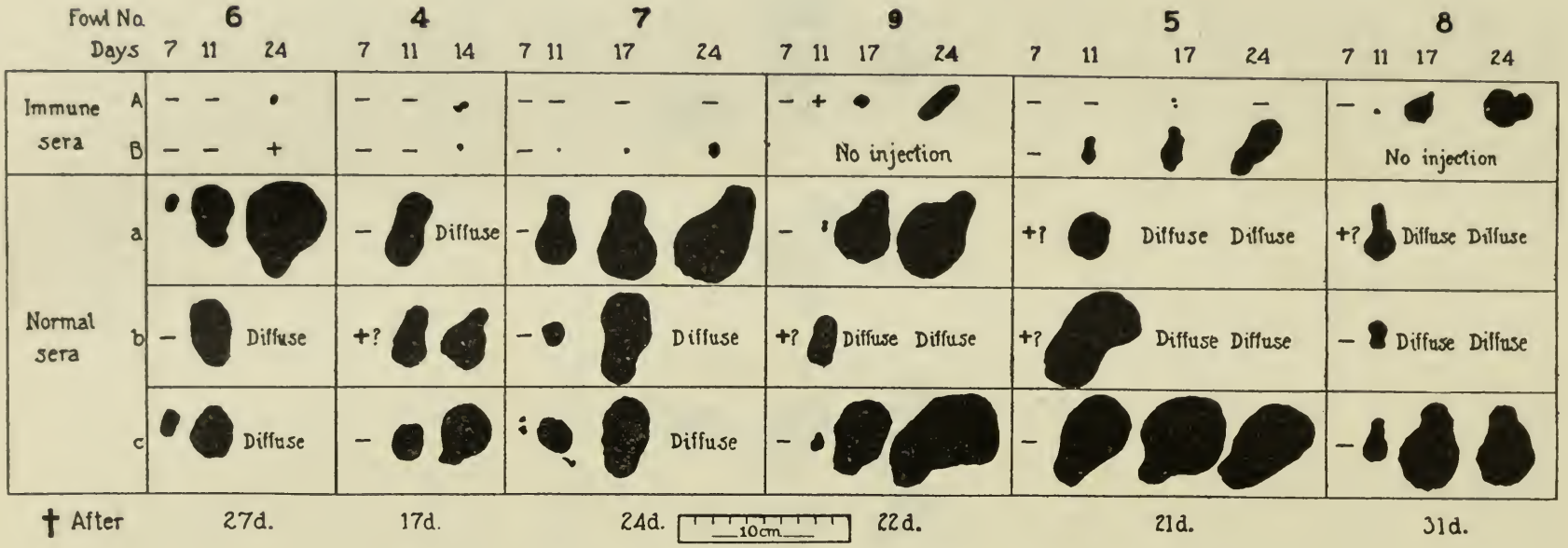
taining the serum of Immune Goose B. The injections were made into the upper wing, upper leg, and pectoral muscles. As Text-figs. 2, 3, and 4 show, large growths rapidly developed where the control mixtures had been placed, whereas none, or only slowly growing ones, were caused by the mixtures containing immune serum.

The neutralizing effect on the tumor-producing agent of the exhausted serum of geese immunized with tumor tissue is clearly shown by these protocols. The agent was especially active in the filtrate used in Experiment 2, as shown by the fact that every one of the fifteen inoculated fowls developed tumors—an occurrence unparalleled in our records. The immune serum completely prevented

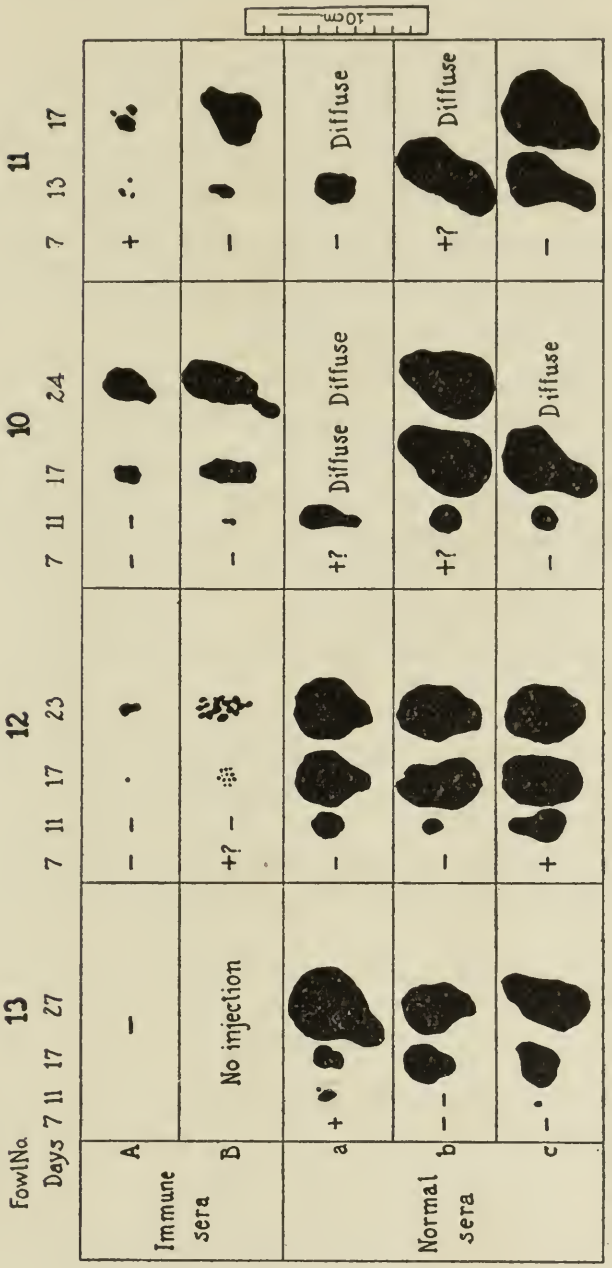



TEXT-FIG. 2. The tumors developing in three fowls receiving mixtures in Proportion X.

tumors at only three injection sites in these fowls, though its protective influence was manifest wherever it had been injected. Very large tumors resulted from all three normal serum mixtures, whence it may be inferred that even the smallest amount of filtrate present in any one, namely that of Proportion X (about 0.66 cc. of filtrate per fowl), contained what might be termed a maximum tumor-producing dose of causative agent. More than twice this amount (1.5 cc. in Proportion Z) yielded tumors that were no larger and grew no more rapidly. The test of the neutralizing power of the immune sera was evidently a severe one in this experiment. In Experiment 1 the filtrate was far less active, as shown by the large proportion of nega-




TEXT-FIG. 3. Tumors in six fowls receiving mixtures in Proportion Y.




TEXT-FIG. 4. Tumors in four fowls receiving mixtures in Proportion Z.

tive fowls (six out of the ten inoculated) and the slow course of the tumors that appeared. Here the neutralization of the tumor-producing agent by the exhausted serum of the immunized geese was complete.

To what is the neutralization referable,—unabsorbed remnants of chicken antibodies? This possibility may be tested by determining whether chicken antibodies as such are able to neutralize the tumor-producing agent. The results with the sera of immunized rabbits gain importance in this connection. For the rabbit sera, while strongly anti-chicken—many times more so than the goose sera—had not the least neutralizing effect on a tumor filtrate.

*Experiment 3.*—A rabbit was given three intravenous injections on successive days of a saline extract of chicken tumor, followed at 6 day intervals by eight intraperitoneal inoculations of a mixture of tumor suspension and citrated blood from fowls moribund of the growth. 8 days after the last injection the animal was bled to death, and its inactivated serum was compared in neutralizing power with that of a normal rabbit. Selective absorption of both was carried out as usual.

| Mixture. | Hemagglutination. |
|---|---|
| 15.5 cc. of rabbit serum + 4 cc. of chicken red blood cells, incubated 1 hr. and serum transferred to 4 cc. of chicken red blood cells, incubated 1 hr..... | Marked.<br>0 |

*Anti-Chicken Titer of the Sera. Hemolysis.*—0.25 cc. of inactivated serum in graded dilutions + 0.25 cc. of 1 in 10 guinea pig complement + 0.25 cc. of chicken red cells.

| Immune serum. | Serum dilution. | | | | | | | | | | | | | | | Guinea pig complement + salt solution + red cells. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | 1/1,024 | 1/2,048 | 1/4,096 | 1/8,192 | 1/16,384 | |
| Untreated.. | C. | C. | C. | C. | C. | C.(?) | Alm. C. | +++ | +++ | +++ | + | ± | Tr. | Tr. | Ft. Tr. | 0 |
| Exhausted.. | + | + | ± | ± | Tr. | Tr. | Tr. | F. test. | 0 | 0 | | | | | | |

Exhaustion was in this instance only approximately complete.

*Hemagglutination.*—The mixtures were the same as those above except that 0.25 cc. of 0.9 per cent salt solution was substituted for guinea pig complement.

| Immune serum. | Serum dilution. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 |
| Untreated..... | +++ | Alm. C. | Alm. C. | C. | C. | ++ | ++ | Tr. | — | 0 |
| Exhausted.... | No agglutination. | | | | | | | | | |

The normal rabbit serum destined to be used in control of the *in vivo* work caused only the slightest hemolysis of chicken cells and no agglutination, when tested prior to its absorption. Thereafter it did not affect the cells at all.

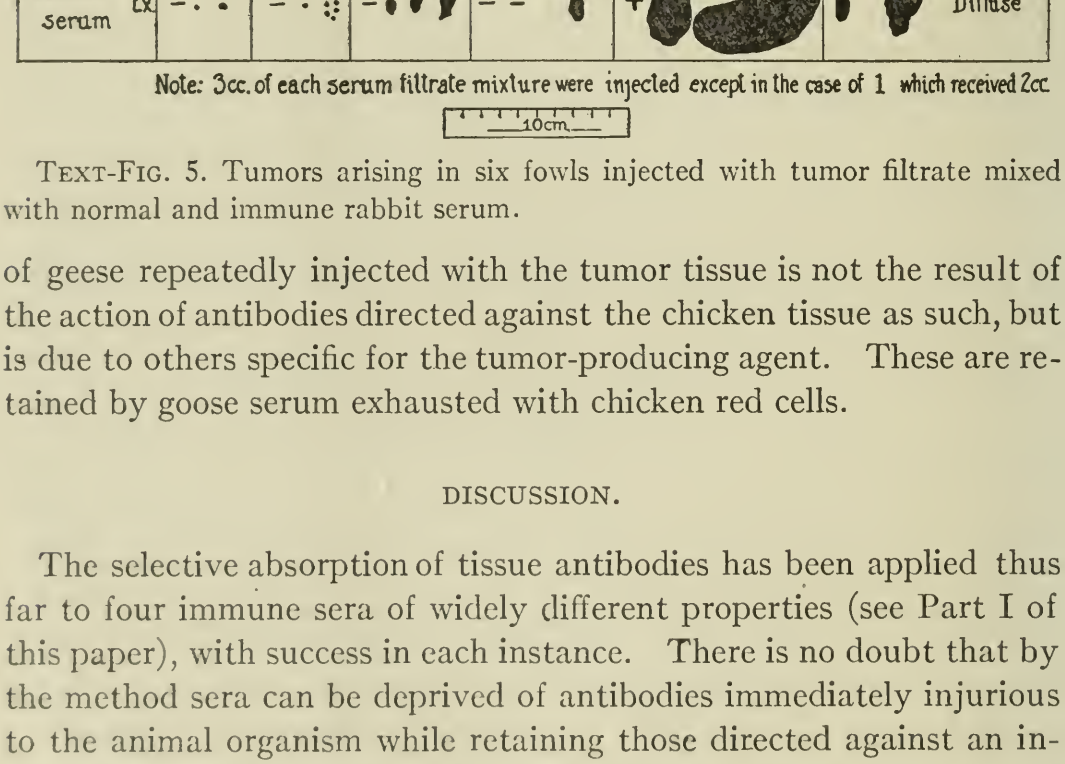
*Precipitation.*—The normal rabbit serum was entirely inactive, but that of the immunized animal caused precipitation when incubated with equal parts of chicken serum diluted up to and including 1 in 2,560.

*In Vivo Tests of Neutralization.*—Three serum specimens were used—normal and immune serum, exhausted as above, and untreated immune serum. A Berkefeld filtrate containing the tumor-producing agent was prepared as usual and mixed with the rabbit sera in the proportion of 6 cc. of filtrate to 12 cc. of serum. Incubation at 38°C. was carried on for 2 hours, cultures were taken, portions of a suspension of diatomaceous earth in salt solution were added to each mixture (0.7 cc. for every 20 cc. of mixture), and injections were made of 3 cc. into five fowls and of 2 cc. into a sixth. In the mixtures with immune serum a floccular precipitate had come down which was distributed by shaking prior to the injections. The sites of injection were varied, as usual. The cultures of the injection fluids were negative after 2 days. Tumors developed in all the fowls, as Text-fig. 5 shows.

The test of the neutralizing power of the rabbit sera was in this case not a severe one. For the late appearance and slow growth of the control tumors clearly showed that no excess of tumor-producing agent was present in the mixtures. Yet there is not the slightest indication of any effect upon the agent of the immune serum, even when it had not been exhausted and was very strong in chicken hemolysin, agglutinin, and precipitin. Said serum had exactly the same effect as serum from a normal rabbit, which contained only the weakest antibodies for the chicken. A floccular precipitation occurred in the mixtures of filtrate and immune serum, but so slowly that it can scarcely have afforded to the tumor-producing agent much protec-

tion from other serum antibodies; and only complete protection by it would explain the results in the inoculated fowls.

This experiment would seem to prove that the neutralization of the tumor-producing agent by the serum of immunized geese is not due to antibodies directed against chicken tissue as such. Such antibodies —or at least those elicited in the immunization of rabbits—fail entirely to injure the tumor-producing agent, even when they are very strong. In view of these facts, the conclusion seems justified that the neutralization of the agent causing a chicken tumor by the serum




TEXT-FIG. 5. Tumors arising in six fowls injected with tumor filtrate mixed with normal and immune rabbit serum.

of geese repeatedly injected with the tumor tissue is not the result of the action of antibodies directed against the chicken tissue as such, but is due to others specific for the tumor-producing agent. These are retained by goose serum exhausted with chicken red cells.

## DISCUSSION.

The selective absorption of tissue antibodies has been applied thus far to four immune sera of widely different properties (see Part I of this paper), with success in each instance. There is no doubt that by the method sera can be deprived of antibodies immediately injurious to the animal organism while retaining those directed against an infectious agent or its products. Applications of the principle in the treatment of disease at once suggest themselves. But many points

must be determined before any practical therapeutic venture is warranted.

First, the late or latent effects on the animal body of exhausted serum must be closely studied. Serum precipitins are not removed with hemolysins and hemagglutinins during the process of exhaustion with red cells. What then is the effect of a specific precipitin acting *in vivo* on an animal of the species against which it is directed? We have been unable to find in the literature a conclusive answer to this obvious question. The controversy over the relation of precipitation to anaphylaxis has resulted in a multitude of *in vivo* experiments, but these have been carried out almost exclusively by introducing precipitin and precipitinogen into animals to which both are alien, or by injecting a serum precipitinogen into an organism that possesses, or will develop, a precipitin for it. Uhlenhuth and Haendel[7] and Doerr and Moldovan[8] have claimed that anti-guinea pig rabbit serum of high precipitin titer is toxic to guinea pigs when injected intravenously; but these authors made no attempt to absorb from the serum the hemolysins and agglutinins present in it and undoubtedly capable of harmful effects. Their work has not been followed up. We plan to do this.

It seems not unlikely that an antiserum resulting from injections of tissues, especially tissues other than blood, will contain elements of possible harm besides hemolysins, hemagglutinins, and precipitins. Here one is confronted with the problem of the specificity of cytotoxins, so long and indecisively debated. Fortunately we are concerned with a single aspect of this problem; namely, that of whether specific cytotoxins, assuming that they exist for the generality of organs—a large assumption—can be removed from serum by its exhaustion with red corpuscles. For should they not be so removable it may be necessary to exhaust a serum with the same kind of tissue employed in the immunization, a matter of much practical difficulty. Experiments on the point with a specific cytotoxic serum, so called, have been begun.

Theoretically the most important use of exhausted sera lies in the treatment of infections of unknown cause. And with each such in-

[7] Uhlenhuth and Haendel, *Z. Immunitätsforsch., Orig.*, 1910, iv, 761.

[8] Doerr, R., and Moldovan, J., *Z. Immunitätsforsch., Orig.*, 1910, vii, 223.

fection two fundamental points would have of necessity to be determined. They are (1) whether the infected tissue will suffice as a practical antigen, and (2) whether the antibodies useful against the infection or its products will survive the serum's exhaustion of antibodies injurious for tissue. The microorganisms in infected tissue employed as antigen will be in many instances in the highest state of pathogenicity. There are advantages to this, but also drawbacks. If the animals to be immunized are themselves susceptible to the infection much less fresh tissue antigen can be employed than of one attenuated by culture or in another way. The dosage of antigen will also be difficult to regulate. Both these obstacles were encountered in Part I of the present work, during our attempts to immunize dogs by injecting them with the blood of rabbits dying of pneumococcus septicemia. So large a percentage of the dogs died that resort was had at length to an antigen of normal tissues and pneumococcus cultures injected separately. The conditions would be much more favorable to successful immunization in the case of infections only slightly pathogenic to the animals employed for immunization. Here tissue containing the infective agent in most virulent form would have great advantages and not improbably decisive ones in the case of cultivable agents that lose their pathogenicity, and incidentally their usefulness as antigen, when grown *in vitro*. Furthermore, it is conceivable that with an agent in highly virulent form so little of the tissue containing it might in certain instances be required as antigen that the serum's titer in elements injurious for tissue would be slight, and the exhaustion in consequence a relatively simple matter.

Little can at this time be said on the persistence of desirable antibodies in an exhausted serum, further than that our experiments make this seem probable in most instances, as do also the observations of others who have used the method of selective absorption to a different end; namely, to demonstrate the specificity of antibodies.[9] Should it become necessary to exhaust a serum of precipitin by means of precipitation in order to render it harmless *in vivo*, even this, it

[9] A noteworthy demonstration of the possibilities of the method is to be found in the work of Todd, C., and White, R. G., *Proc. Roy. Soc. London, Series B*, 1910, lxxxii, 416. By the selective absorption of induced isohemolysins these authors were enabled to recognize the red corpuscles of individual oxen.

would seem, might be done without, in most instances, removing the antibodies directed against an infectious agent. For Gay and Stone[10] have made many attempts to bring down such elements in a serum precipitate, but without success.

Although the use of exhausted serum in the treatment of infectious diseases is at present but a distant possibility, there lies open a field for its immediate employment. Through the method of absorption much may be learnt regarding serum immunity to animal diseases—as witness the case of the chicken sarcoma,—and to human infections of unknown cause that are transmissible to animals. For the tissues of infected animals will furnish a ready antigen for experimental purposes, while normal individuals of the same species can be used as test objects to determine whether the exhausted sera resulting from immunization possess any protective power. A concrete illustration of such a possibility is afforded by some recent work of Nicolle and Blaizot.[11] These authors state that they have produced an effective antityphus serum in donkeys by injection with the spleens of guinea pigs dying of the disease. The serum is intended for use in human beings, but they find that with it guinea pigs can be cured of typhus, though the serum is so toxic for such animals that it can be given only in small quantities, which hinders the tests. It would have been interesting to deprive the serum of this toxicity by selective absorption with guinea pig cells, with a view to a more striking demonstration of its antityphus power.

## SUMMARY.

By the method of selective absorption with tissue, protective serum antibodies have been demonstrated in the case of an infection of unknown cause; namely, a chicken sarcoma transmitted by a filterable agent. Geese were repeatedly injected with the finely ground sarcoma and with blood from fowls moribund of it; and their sera acquired the power to prevent the tumor-producing agent from causing growths. That this was not due to antibodies elicited by the chicken tissue as such was shown by exhaustion of the goose sera with chicken

[10] Gay, F. P., and Stone, R. L., *J. Immunol.*, 1916, i, 83.

[11] Nicolle, C., and Blaizot, L., *Ann. Inst. Pasteur*, 1916, xxx, 446.

red cells, a step which had not the least effect on the tumor-preventing power, and also by experiments with rabbits immunized as were the geese. These animals developed strong chicken antibodies in their sera which failed nevertheless to affect the tumor-producing agent.

Serum immunity to the chicken sarcoma is weak at best; and in the case of some other infections of unknown cause, more striking results may be anticipated from the method of selective absorption. It is even conceivable that by its means sera of therapeutic usefulness may become available. But much remains to be settled as regards the dangers of exhausted sera and the limitations of the method. Fortunately there exists an immediate field for the latter in laboratory studies on the nature of immunity to infections of which the cause has not been recognized.

# THE EFFECT OF CARBON DIOXIDE IN THE CULTIVATION OF THE MENINGOCOCCUS.

By FREDERICK L. GATES, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 15 and 16.

(Received for publication, January 29, 1919.)

The experiments here reported are the outcome of observations made in applying the recommendation of Cohen and his associates[1,2,3] that meningococci be grown at a partial oxygen tension, obtained by substituting carbon dioxide for approximately 10 per cent of the air in a closed container.

The moisture requirement of the meningococcus has been repeatedly emphasized,[4] and it seemed important to determine whether Cohen's results might not be due, in part at least, to the retention of moisture in the partial tension chambers. In our series of carrier examinations two sets of carrier plates were incubated in moist chambers; one set in air, the other in the presence of carbon dioxide, from 2 to 25 per cent by volume. Our uniform experience has been that nasopharyngeal strains of meningococci grow as well, or better, in air saturated with water vapor in a closed chamber as in a similarly saturated atmosphere containing a small percentage of carbon dioxide. The conditions governing the luxuriance of the growth are factors of the humidity of the chamber and the qualities of the medium, and not of the slight differences in oxygen tension resulting from the replacement of a small part of the air with carbon dioxide.

Recently the opportunity presented itself at Camp Zachary Taylor of making similar comparisons with growths of meningococci

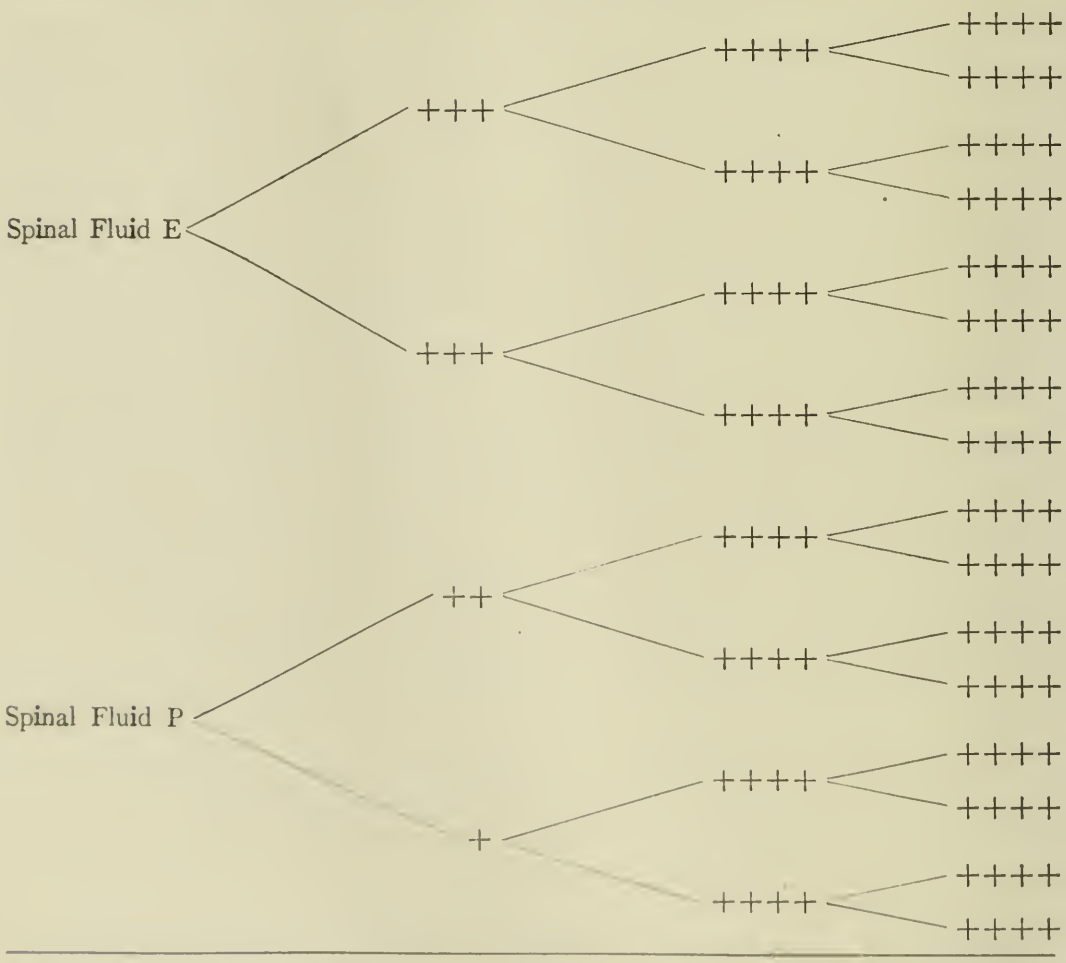
[1] Cohen, M. B., and Markle, L., A method which greatly facilitates the culture of the meningococcus, *J. Am. Med. Assn.*, 1916, lxvii, 1302.

[2] Cohen, M. B., Cultivation of the meningococcus under partial oxygen tension, *J. Am. Med. Assn.*, 1918, lxx, 1999.

[3] Cohen, M. B., and Fleming, J. S., The diagnosis of epidemic meningitis and the control of its treatment by rapid bacteriologic and serologic methods, *J. Infect. Dis.*, 1918, xxiii, 337.

[4] Lloyd, D. J., On vitamines, amino-acids, and other chemical factors involved in the growth of the meningococcus, *J. Path. and Bacteriol.*, 1916–17, xxi, 113.

from spinal fluids. As a routine approximately 2 cc. of spinal fluid were added to each of six slants of medium.[5] Three tubes of each set were incubated in a water-sealed chamber, in air, while three were incubated in a similar chamber containing 10 per cent carbon dioxide. No significant difference could be observed in the growths under these conditions. The slight differences noted favored the chamber containing air alone. Successive generations were also grown alternately in air and in 10 per cent carbon dioxide, without visible diminution in luxuriance. Two typical strains may be charted by the scheme by which Cohen has illustrated his results. Upstrokes indicate cultures in air, downstrokes cultures in 10 per cent carbon dioxide. One plus sign indicates a single colony, two indicate several colonies, three many, and four a confluent growth.




[5] Veal infusion agar, with glucose 1 per cent, adjusted to pH 7.4, to which 5 per cent sterile, unheated, citrated horse plasma was added just before tubing. This is a medium highly favorable to the meningococcus.

From these and similar findings the conclusion was reached that the replacement of 10 per cent of air by carbon dioxide has no appreciable effect on the growth of either nasopharyngeal or spinal strains of meningococci, under the conditions of our experiments.

Cohen considers that the effect of carbon dioxide, as he describes it, is not specific for this gas: that it acts merely as a neutral agent, displacing oxygen, and he calls the meningococcus a "microaerophile." A brief consideration of the composition of air, and of the actual displacement of oxygen by 10 per cent carbon dioxide shows that only approximately 2 per cent of oxygen would be displaced and its concentration reduced from 20 per cent to 18 per cent. Such a slight diminution could hardly be expected to produce the marked effects that Cohen reports. Moreover, if the action of carbon dioxide were not specific, results similar to Cohen's should be obtained with any neutral gas, such as nitrogen, which is chemically so inert. The fact that air is composed largely of nitrogen, and that only peculiar species of bacteria are able to utilize nitrogen from the air would seem to make it the most desirable agent for replacing oxygen in testing various oxygen tensions. Therefore nitrogen was used to replace oxygen in the following experiment.

*Experiment 1.*—January 13, 1919.

Eight plates of veal infusion agar, pH 7.4, with 5 per cent fresh sterile rabbit serum were inoculated with a suspension of a recently isolated spinal strain of normal meningococcus. Two of these plates were placed in each of four large Novy jars containing a little water. In three of the jars concentrations of nitrogen of 10, 25, and 50 per cent, in addition to that already present in the air, were obtained by partial exhaustion of the air and replacement with the gas from a tank. Thus partial oxygen tensions of approximately 18, 15, and 10 per cent were produced in the jars. The fourth jar served as an air control.

After 16 hours incubation the growths of meningococcus were profuse and practically identical in air, in 18 per cent oxygen, and in 15 per cent oxygen. Luxuriant confluent streaks of organisms showed the path of the inoculation. On one of the plates in 10 per cent oxygen the growth was poor, in the other very poor. Only a few small confluent masses of growth showed the site of heaviest inoculation. A plate from each jar is reproduced in Plate 15, Petri Plates 5 to 8.

The meningococcus did not thrive on artificial medium when deprived of half its accustomed oxygen tension. But this experiment does

not indicate that variations of 2 to 5 per cent below normal make any essential difference in its growth. What effect would increased oxygen tension have?

*Experiment 2.*—January 14, 1919.

Plates similar to those used in Experiment 1 and inoculated as before were divided among the four Novy jars. In a similar manner oxygen was used to replace the exhausted air in percentages of 5, 15, and 25, making the oxygen tensions of the atmospheres in the jars approximately 24, 32, and 40 per cent. The fourth jar again contained air alone.

After 16 hours incubation the growths in all the jars were profuse. Beside the confluent growths where the plates were most heavily inoculated large single colonies of luxuriantly growing organisms appeared. The meningococcus grew as well in 40 per cent oxygen as in air (Plate 15, Petri Plates 1 to 4).

Provided the oxygen tension is sufficient to support its growth, the meningococcus does not seem to be exacting about the concentration of its oxygen supply. These experiments show that the effects of increased carbon dioxide tension, as described by Cohen, are not due to the displacement of oxygen and that the meningococcus is not a "microaerophile." The action of carbon dioxide must be inherent in some specific property of the gas. Carbon dioxide in solution acts as a weak acid and might have a chemical effect on the medium in which meningococci are grown and on the organisms themselves. If serum agar plates, such as are used for isolating the meningococcus, are incubated in a moist atmosphere containing 10 to 30 per cent carbon dioxide, their reaction may be shifted toward the acid end of the pH scale. The following experiment shows this reaction and its effect upon a recently isolated (fourth generation) spinal strain of a normal meningococcus.

*Experiment 3.*—January 3, 1919.

Four 100 cc. lots of veal infusion agar were adjusted to the following reactions.

| | Titratable acidity (phenolphthalein). *per cent* | pH concentration (phenolsulfonephthalein). |
|---|---|---|
| Lot 1 | 1.6 | 7.2 |
| " 2 | 0.8 | 7.8 |
| " 3 | 0.4 | 7.9 |
| " 4 | 0.3 | 8.0+ |

To each lot were added 5 cc. of sterile, unheated rabbit serum, and eight plates were poured. Four plates of each set were heavily inoculated with a

suspension of meningococci from a third generation slant of a spinal strain. Eight plates (one inoculated and one sterile from each lot) were placed in each of four large Novy jars. In three of the jars concentrations of carbon dioxide of 10, 20, and 30 per cent were obtained by partial exhaustion of the air and replacement with the gas from a tank. The fourth jar served as an air control. After 18 hours incubation at 37.5°C. the growths of meningococci were noted and the pH concentrations of the uninoculated plates read with phenolsulfonephthalein and cresol purple as the indicators.

TABLE I.

| Set No. | Original titer. | | Final titer (pH). | | | | Growths. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Acidity. | pH | Air. | Carbon dioxide. | | | Air. | Carbon dioxide. | | |
| | | | | 10 per cent. | 20 per cent. | 30 per cent. | | 10 per cent. | 20 per cent. | 30 per cent. |
| | *per cent* | | | | | | | | | |
| 1 | 1.6 | 7.2 | 7.4 | 7.0 | 6.7 | 6.4 | Profuse. | Profuse. | Profuse. | Fair. |
| 2 | 0.8 | 7.8 | 8.0 | 7.4 | 6.8 | 6.6 | Good. | " | " | " |
| 3 | 0.4 | 7.9 | 8+ | 7.4 | 6.8 | 6.6 | None. | " | " | Good. |
| 4 | 0.3 | 8+ | 8+ | 7.6 | 6.8 | 6.7 | " | " | " | Profuse. |

Table I shows the results, and Plate 16 the growths obtained in air, in 10 per cent carbon dioxide, and in 30 per cent carbon dioxide. The growths in 10 and 20 per cent carbon dioxide were practically identical. Several points are to be noted in an analysis of these findings.

The meningococcus does not grow in a medium of a titratable acidity of +0.4 or less when this corresponds to a pH value greater than 7.8 to 8.0 on the Sörensen scale. It may grow luxuriantly on a medium with a titratable acidity of +1.6 when this corresponds to pH 7.2. The fallibility of the old method of titrating media is clearly seen.

The partial saturation of a medium with carbon dioxide from an atmosphere containing 10 to 30 per cent of the gas may increase its hydrogen ion concentration markedly. Almost regardless of the original hydrogen ion concentration, the final reaction seems to represent a state of equilibrium between the carbon dioxide in the medium and in the surrounding air. Thus in the present experiment 10 per cent carbon dioxide brought the more alkaline plates to ap-

proximately pH 7.4 to 7.6, 20 per cent brought them to pH 6.8, and 30 per cent brought them to pH 6.6 to 6.7.

It is evident that such an action of carbon dioxide on the medium might exert either a favorable or an unfavorable effect upon meningococci, according to the original reaction of the medium. A favorable effect is seen in Petri Plates 3 and 4 of all the carbon dioxide series—a medium too alkaline to support growth was brought to a hydrogen ion concentration favorable to the growth of the meningococcus. An unfavorable effect, on the other hand, is seen in Petri Plates 1 and 2 of the carbon dioxide 30 per cent series; the medium was rendered too acid, and the meningococcus grew less well than in air.

Cohen's contention that meningococci which have developed aerobically do not grow well in the presence of carbon dioxide and *vice versa* is not borne out by this experiment, in which a strain isolated and grown for three generations aerobically grows well in carbon dioxide on a medium in which it cannot grow in air. In connection with the first experiment it may be pointed out that a meningococcus isolated and subcultured in air grew equally well under slightly reduced oxygen tension.

Cohen's reports indicate that this effect of carbon dioxide on the medium has operated favorably in his experiments, and thus the discrepancy between his results and ours may be explained. He and Fleming[3] describe the use of media 0.2 to 0.3 per cent acid to phenolphthalein, under the impression "that the meningococcus will not usually grow when the reaction is over plus 0.5 [per cent] acid to phenolphthalein." In serum agar, 0.2 to 0.3 per cent acid usually corresponds to pH 8.2 to 7.8 on the Sörensen scale, according to the amount of buffer present. Kligler[6] has recently found that the range of growth of the meningococcus in serum dextrose broth is pH 6.1 to 7.8, with the optimum at pH 7.4, and this fact, in conjunction with the effect of carbon dioxide in shifting the reaction toward the acid side, as shown above, suggests that incubation in a partial tension of carbon dioxide has made Cohen's media less unfavorable to the meningococcus, and so, combined with the undoubted aid of

[6] Kligler, I. J., personal communication.

moisture in the closed container, produced the results that he describes.

As for the effect of carbon dioxide on the organisms themselves, a specific action is not so easily demonstrated. That partial tensions up to 30 per cent may not be injurious is shown by the growths on the less acid plates of this series. On the other hand, Shaw-MacKenzie[7] states that carbon dioxide, 22 volumes per cent in Ringer-Locke solution, causes the death of the meningococcus in 20 minutes and suggests that "even the $CO_2$ normally occurring in the plasma and body fluids may form part of the protective processes of the body."

## SUMMARY.

The meningococcus is not a "microaerophile." It grows equally well in atmospheres containing from 15 to 40 per cent oxygen.

If small amounts of carbon dioxide affect the growth of the meningococcus on an artificial medium it is by changing the reaction of the medium, not by slightly reducing the oxygen tension of the surrounding air.

The fallibility of titrating the total acidity of a medium is again clearly demonstrated. A reaction favorable to the meningococcus cannot be determined from the total titratable acidity but depends solely upon the hydrogen ion concentration of the medium. The optimum for the meningococcus is approximately at pH 7.4.

The value of a moist chamber in the cultivation of the meningococcus is shown by unusually luxuriant growth when other conditions are also favorable.

## *Addendum.*

Since the completion of these experiments and the receipt of this paper for publication, St. John (St. John, J. H., *Med. Rec.*, 1919, xcv, 184) has reported his experiments on "Oxygen tension in its relation to the meningococcus." He concludes that the oxygen tension factor is at least of minor importance in comparison with the effect of moisture in promoting the growth of meningococci. He calls

[7] Shaw-MacKenzie, J. A., Toxic action of carbonic and other weak acids on the meningococcus, *J. Roy. Army Med. Corps*, 1918, xxxi, 1.

attention to the fact that the meningococcus is known to be especially sensitive to the degree of moisture and the reaction of the medium, and suggests that "the reaction of the meningococcus medium may be favorably influenced by certain gases evolved by the growing *B. subtilis*, but this consideration is also minimized by comparison with moisture controls."

At the time that St. John's article appeared there was in press a paper by Frederick L. Gates and Edgar T. H. Tsen on "The effect of moisture on the growth of the meningococcus" which was to have appeared in this number of *The Journal of Experimental Medicine*. Our findings on the importance of moisture in the cultivation of the meningococcus merely corroborated St. John's, and added nothing but emphasis to his conclusions. Our paper was therefore withdrawn from publication. We feel, however, that the degree of humidity most favorable to the meningococcus is not to be obtained easily unless closed containers are employed.

## EXPLANATION OF PLATES.

### Plate 15.

The effect of different oxygen tensions on the growth of the meningococcus.

Petri Plates 1 to 3. 16 hour growths of a spinal strain under increased oxygen tension. Petri Plate 4. A control growth in air.

Petri Plate 5. A control growth in air. Petri Plates 6 to 8. 16 hour growths of a spinal strain under decreased oxygen tension.

### Plate 16.

The effect of carbon dioxide on the growth of the meningococcus, caused by changing the hydrogen ion concentration of the medium.

Petri Plates 1, 1, 1. Original pH 7.2; titratable acidity 1.6 per cent.

Petri Plates 2, 2, 2. Original pH 7.8; titratable acidity 0.8 per cent.

Petri Plates 3, 3, 3. Original pH 7.9; titratable acidity 0.4 per cent.

Petri Plates 4, 4, 4. Original pH 8+; titratable acidity 0.3 per cent.

In Sets 1 and 2, 30 per cent carbon dioxide increased the hydrogen ion concentration beyond the zone favorable to growth. In Sets 3 and 4, 10 and 30 per cent carbon dioxide brought plates originally too alkaline to support growth into the zone of hydrogen ion concentration favorable to the growth of the meningococcus.

# CICATRIZATION OF WOUNDS.

## X. A General Equation for the Law of Cicatrization of Surface Wounds.

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It has been shown[1] by the extrapolation formula

$$S_n = S_{n-1}\left[1 - i\left(t + \sqrt{t + nt}\right)\right]$$

that the normal progress of cicatrization of surface wounds follows a definite curve. The fact that many biological and chemical phenomena are expressed by exponential formulas suggested the comparison, if possible, of the curve for the cicatrization of wounds with other curves expressing biological phenomena. It is well known that the exponential function plays an important part in natural phenomena. It expresses the general law called by Lord Kelvin "the compound interest law," and by Mellor, the "ubiquitous law."

I had already studied a formula of the form

$$y = \frac{K}{x - a} \quad \text{(hyperbola)}$$

which was suggested to me by Professor Houssay, by means of which he expresses the phenomenon of regression of certain organs in animals, under special conditions; but this proved to be unsuccessful.

On the basis that during the short time $dt$ the cicatrized area $ds$ remains proportional to the total area, we can write

$$-ds = KSdt \tag{1}$$

[1] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451, 461; 1917, xxv, 721.

by integration in respect to time,

$$T = -\int_{S_o}^{S} \frac{ds}{KS} \tag{2}$$

or

$$T = -\frac{1}{K}\int_{S_o}^{S} \frac{ds}{S}$$

hence

$$T = \frac{1}{K}\ \mathrm{Log}_e \frac{S_o}{S}$$

which is similar to the equation of Slater,[2]

$$T = \frac{1}{K}\ \mathrm{Log}_e \frac{N+n}{N}$$

and finally,

$$KT = \mathrm{Log}_e \frac{S_o}{S} \tag{3}$$

that is,

$$S = S_o e^{-KT}$$

We can then compute the values of the coefficient $K$ for the different values of $T$. $K$ increases regularly. Therefore, the curve obtained from the equation

$$S = S_o e^{-KT}$$

does not correspond to the facts, and gives for every value of $T$ a certain value of $S$ which deviates more and more from that calculated according to formula (1) (extrapolation form). We were then obliged to introduce a new coefficient, stating the problem in the following way: Is it better to attempt to find this new coefficient by giving to $T$ its real value and by studying the variations of $K$, or is it more advisable to study the variations of the exponent if $K$ remains

[2] Slater, A., *Biochem. J.*, 1912–13, vii, 197.

constant; that is, the variations of a certain coefficient $\alpha$ as in the exponent

$$-K(T+\alpha) \tag{4}$$

The study of a large number of cases showed that by trying to find the correction of the coefficient $K$, I encountered a practical difficulty from the fact that since this coefficient is small in respect to $T$, the smallest numerical variations such as those arising from calculation errors with 2 or 3 decimal numbers were of sufficient importance to destroy the concordance of the curves. On the contrary, in the second case, fairly important variations in a certain coefficient $K_2$, the connection of which with $\alpha$ can be expressed as

$$\alpha = \frac{T^2}{K_2}, \tag{5}$$

interfered very little with the accuracy of the calculation.

Text-fig. 1 shows the variations of the coefficient $K$ in function of time. The angular coefficient of the lines seems to vary proportionally with the index of cicatrization, as

$$\text{index } i = \frac{S_o - S}{S_o\,(t + \sqrt{t})}$$

It is by no means certain that these lines are straight lines mathematically (see the straight dotted line in Text-fig. 1), but the observations are limited by time and it is difficult to determine this point. In this chart the value of $K$ is given by equation (3) from which the following formula is obtained:

$$K = \frac{\text{Log } S_o - \text{Log } S}{T} \tag{6}$$

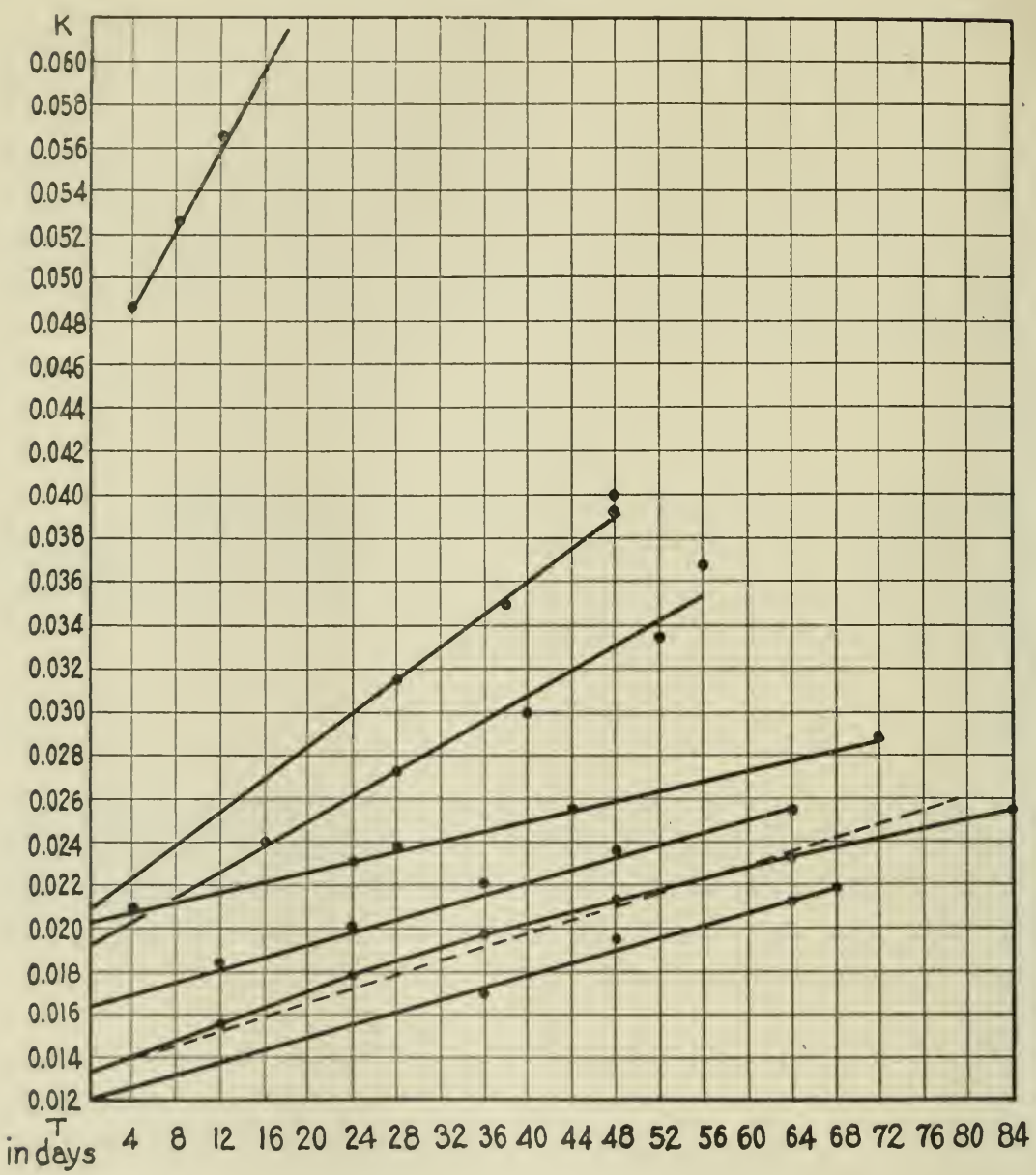
Text-fig. 2, on the contrary, shows the variations of the coefficient $\alpha$ previously determined, the value of which is

$$\alpha = \frac{\text{Log } S_o - \text{Log } S}{K} - T \tag{7}$$

By plotting in ordinates the values of $\alpha$ which represent the difference between the curve resulting from equation (3) and that resulting

from equation (1), we obtain a curve which expresses the law of these differences. It is a branch of parabola and the equation is

$$y^2 = 2\ px$$




TEXT-FIG. 1. Variations of the coefficient $K$, in function of time.

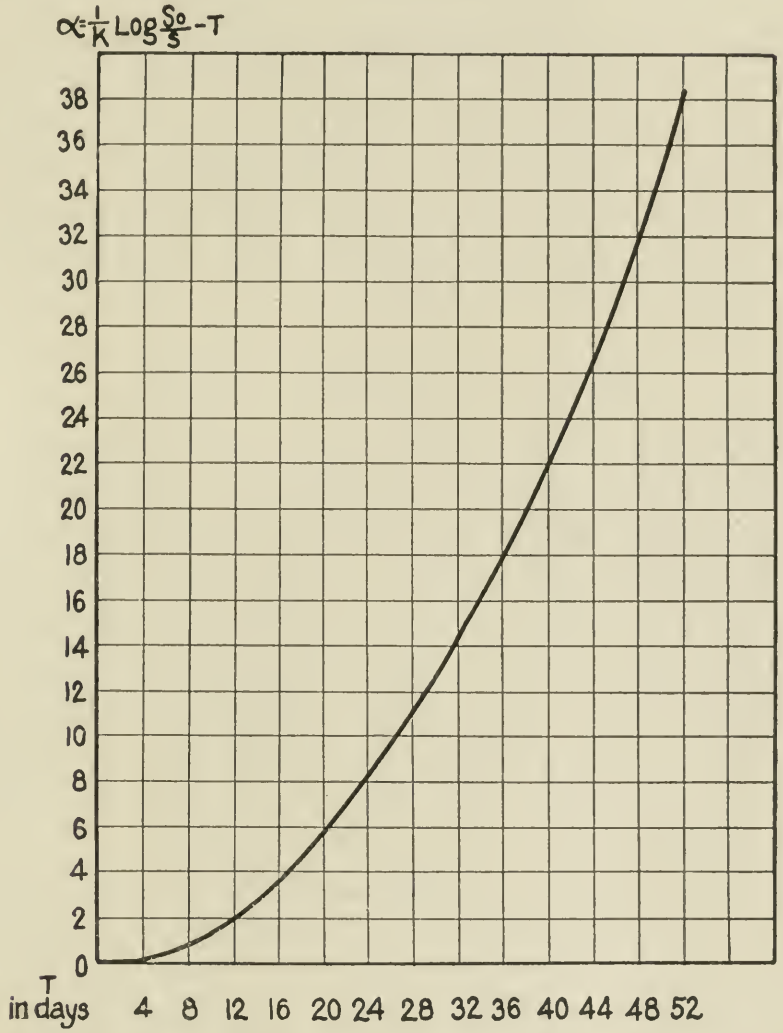
That is, by replacing the letters by those we have adopted, *viz.*, $y = T$, and $x = \alpha$,

(8) $$\alpha = \frac{T^2}{2p}$$

The significance of the coefficient $K_2$ in equation (5) appears now clearly, and the equation may be written

$$S_T = S_o e^{-K\left(T+\frac{T^2}{2p}\right)} \tag{9}$$

which is the general equation of the law.




TEXT-FIG. 2. Variations of the coefficient $\alpha$ in function of time in the formula

$$S_T = S_o e^{-K(T+\alpha)}$$

Before we begin a thorough study of the coefficients it may be interesting to compare, for example, two series of figures representing the ordinates, *viz.* the areas of wounds in square centimeters, of two

cicatrization curves obtained, the first (figures of the upper row) by means of the last exponential equation (9), the second (figures of the lower row) by means of the former extrapolation formula (1). It is obvious that the concordance is almost perfect and that the differences are beyond the errors of experimentation (Table I).

These two examples suffice to show that the proposed equation fulfills the required conditions; in all the cases the coincidence is equally satisfactory. Slight differences, however, sometimes may be observed at the beginning of the curve (for $T = 4, 8, 12$ days), but since the exponential equation has been mathematically studied in a different manner from the first formula, and since, on the other hand, these differences may be affected by errors of measure of the area of wounds, it cannot be concluded that the equation previously proposed is more accurate than the new one.

*Study of the Coefficients K and 2p.*

As the coefficient $K$ can be determined within 4 days, that is from two points on the curve, 4 days apart, and as the contraction, especially for the large wounds, plays the principal part at the beginning of cicatrization, this coefficient characterizes the contraction, and during the first days the relative rate of repair, with reference to the total area of the wound. But it has been stated[1] that this rate is itself a function of the age of the man, within certain limits. Hence the coefficient $K$ must logically be proportional to the index of cicatrization $i$ which plays the same part in formula (1). The calculation of a number of curves shows that this is so.

The velocity of repair is originally determined by the area of the wound. We have assumed that at the beginning of the phenomenon it remained proportional to the area for a very short time. We proceeded from this assumption to state the differential equation

$$-ds = KSdt$$

If the velocity remained proportional to the area, this would explain the increasing delay due to the reduction of the area of the wound. On account of this delay, the phenomenon is expressed by a logarithmic curve and not by a straight line, for, at a certain moment $T$, the area which is not yet cicatrized is $TM = S$ (Text-fig. 3).

TABLE I.

| Area calculated with. | Time in days. | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 | 52 | 56 | 60 | 64 | 68 | 72 | 76 |
| **Patient 263.** | | | | | | | | | | | | | | | | | | | | |
| | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| Equation (9) | 61.8 | 50.8 | 41.4 | 33.4 | 26.8 | 21.2 | 16.7 | 13.1 | 10.2 | 7.7 | 6.0 | 4.5 | 3.4 | 2.5 | 1.8 | 1.4 | 1.0 | 0.7 | 0.5 | 0.4 |
| " (1) | 61.8 | 51.0 | 41.6 | 33.6 | 26.9 | 21.3 | 16.7 | 13.1 | 10.2 | 7.7 | 6.0 | 4.5 | 3.4 | 2.5 | 1.8 | 1.4 | 1.0 | 0.7 | 0.5 | 0.4 |
| **Patient 706.** | | | | | | | | | | | | | | | | | | | | |
| Equation (9) | 27.4 | 21.8 | 17.1 | 13.4 | 9.9 | 7.2 | 5.1 | 3.6 | 2.4 | 1.6 | 1.1 | 0.69 | 0.43 | | | | | | | |
| " (1) | 27.4 | 22.0 | 17.1 | 13.0 | 9.6 | 7.2 | 5.1 | 3.6 | 2.4 | 1.6 | 1.1 | 0.7 | 0.46 | | | | | | | |

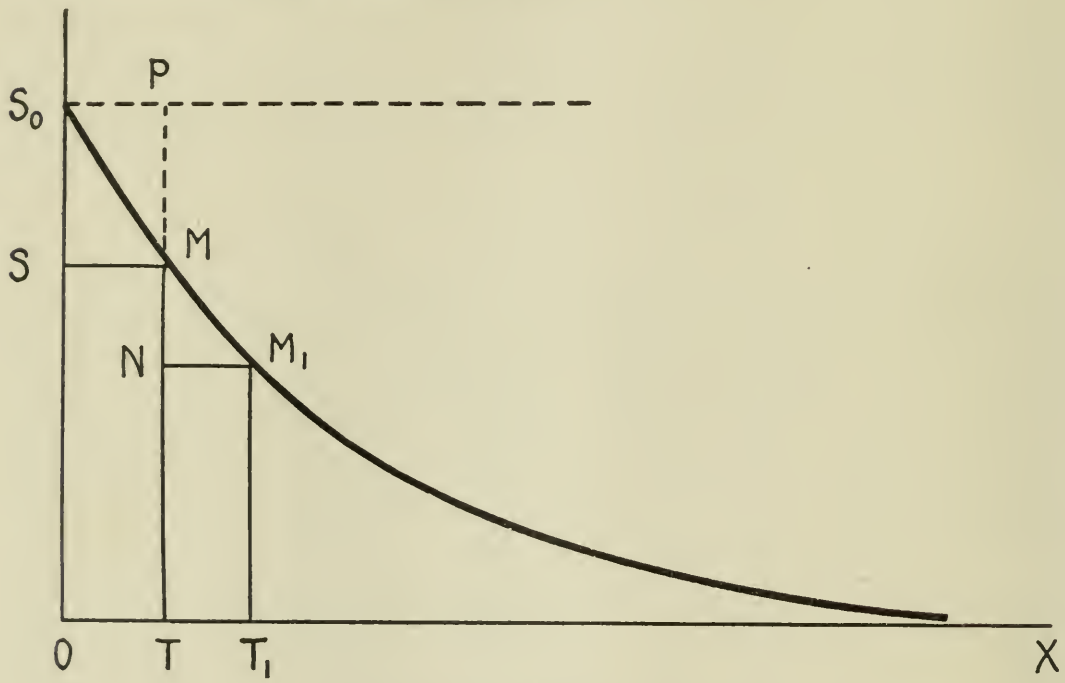
The surface which is already cicatrized is represented by

$$MP = S_o - S$$

The law of the curve, if logarithmic, is that the decrease $MN$ of the ordinate $S$, when passing from the time $T$ to the time $T_1$, is proportional to the length $S$ of the ordinate; that is, the area cicatrized during the time $T_1 - T$ is proportional to the area which is not yet cicatrized. This is what we have written in mathematical symbols, for infinitesimal values

$$-ds = KSdt$$

for $ds$ corresponds to $MN$ and $dt$ to $T_1 - T$.




TEXT-FIG. 3. Logarithmic curve.

But this hypothesis, true at the beginning of the phenomenon, under certain conditions, grows rapidly erroneous, since we have stated that the curve resulting from this equation deviates more and more from the experimental facts. Hence the diminution of the area is not the only factor which governs the real curve. A careful study of the latter and a comparison with the plain logarithmic curve shows that to the *decreasing* acceleration a uniformly *increasing* acceleration is opposed, which at every moment counteracts the effect of the delay due to the decrease of the area.

But if the hypothesis is justified at a certain moment, the simple equation which proceeds from it

$$S_T = S_o e^{-KT}$$

must represent the phenomenon at the beginning and must express the part played by the first factor, the contraction, which intervenes alone at this moment, as long as the second disturbing factor does not enter into action, or its part is small with reference to that of the first one.

We can verify the correctness of this statement by drawing the curve representing the contraction of a wound; this can be done by measuring the total area of the new scar tissue, no longer merely the area of granulations. For we know that the decrease of this area measures solely the contraction.[3] Then the contraction curve obtained in this way should logically, within certain limits, comply with the law expressed by equation (3). Text-fig. 4 illustrates this fact, and our first hypothesis was therefore justifiable.

It is easily seen that the phenomenon follows the law until, owing to the decrease of the wound area, a more important part of the work of reparation in respect to the area of the granular surface is carried out by the second factor. Then, the decrease of the area being much greater than indicated by equation (3), the contraction, which depends obviously on the area not yet cicatrized, slackens gradually, until it ceases entirely. These observations would show plainly, if we were not already aware of it, that the second factor is the epithelization, and it is then understood that its action is represented in equation (9) by the quotient $\frac{T^2}{2p}$, which expresses that its efficiency, feeble at the beginning, increases slowly at first, then more rapidly, according to a parabolic law.

The above statements are generally verified only if the first observations are made when the cicatrization has already begun, and little or no epithelization has yet appeared. The starting-points of both curves (contraction and cicatrization) are confounded, that is they have the same ordinate at the time 0, so that the surface of the wound itself and that of the cicatrix cannot be discerned from each

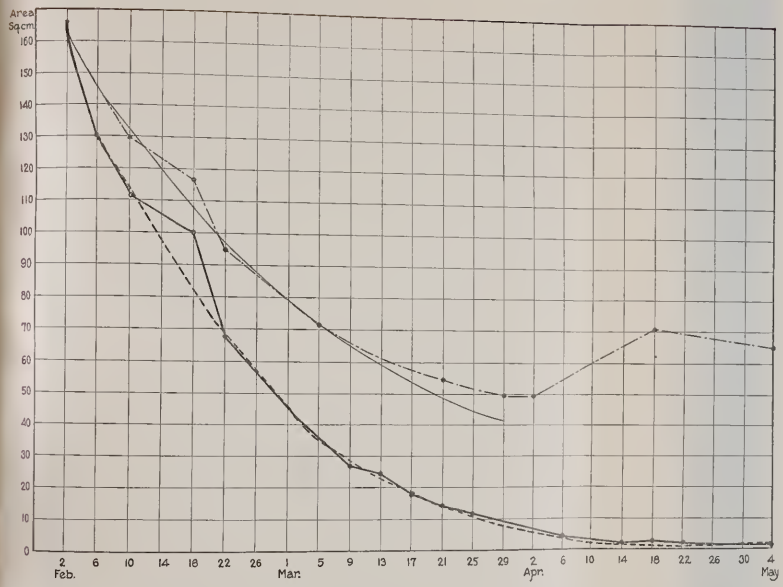
[3] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

other, the edges of the wound being constituted by the old skin or a new and hardly visible epithelial border (Text-fig. 4). When epidermization has already begun, the ordinates at the time 0 are not coincident. If $S_o$ is the ordinate of the area of granulations, in square centimeters, and $S_1$ the area of the cicatrix, let us call $A$ the difference $S_o - S_1$. $A$ represents the surface already covered by the new epithelium, and the equation becomes

$$S_1 = A + S_o e^{-KT}$$

$A + S_o$ is what we have called the area of the cicatrix. But in this case it is often more difficult to verify formula (3) for the contraction, because the epithelization may have become important enough to disturb the simple phenomenon of contraction, the disturbing action being obviously the function of $A$. The difficult definition of the outline of the cicatrix is also a cause of error. This explains why it is difficult, except on experimental wounds, to find cases on which observation can be made accurately. However, Text-fig. 5 shows that this is possible. The measure of the cicatrix area is made by drawing on cellophane the common limit of the old skin and of the new epithelium, or scar tissue. It is essential to draw this outline on the skin itself, in order to prevent errors of interpretation and of drawing which are frequent, as this common limit often lacks sharpness. But if at the beginning it is tattooed (on animals) or drawn with a dermographic pencil (on men), the measures become comparable and can be done with sufficient accuracy. Every time a drawing is taken, it is advisable to go over the outline again with the pencil where it shows a tendency to be obliterated.

As regards the term $\frac{T^2}{2p}$, what has already been said concerning its growing action in function of time must be taken merely from a mathematical standpoint and not as an assumption dealing with the mechanism of the phenomenon itself. The activities of the real factors are not known, and we can only measure one of the results of these activities, which may vary proportionally to the mathematical factors. Our knowledge does not go beyond that. For example, we know that $\frac{T^2}{2p}$ increases slowly at first, then rapidly, and we assume




TEXT-FIG. 4. Patient 360. The dotted and broken line represents the contraction of the wound (for details of technique see Carrel and Hartmann[3]). The light line is the calculated curve, according to the formula

$$S_T = S_{0^c} - KT$$

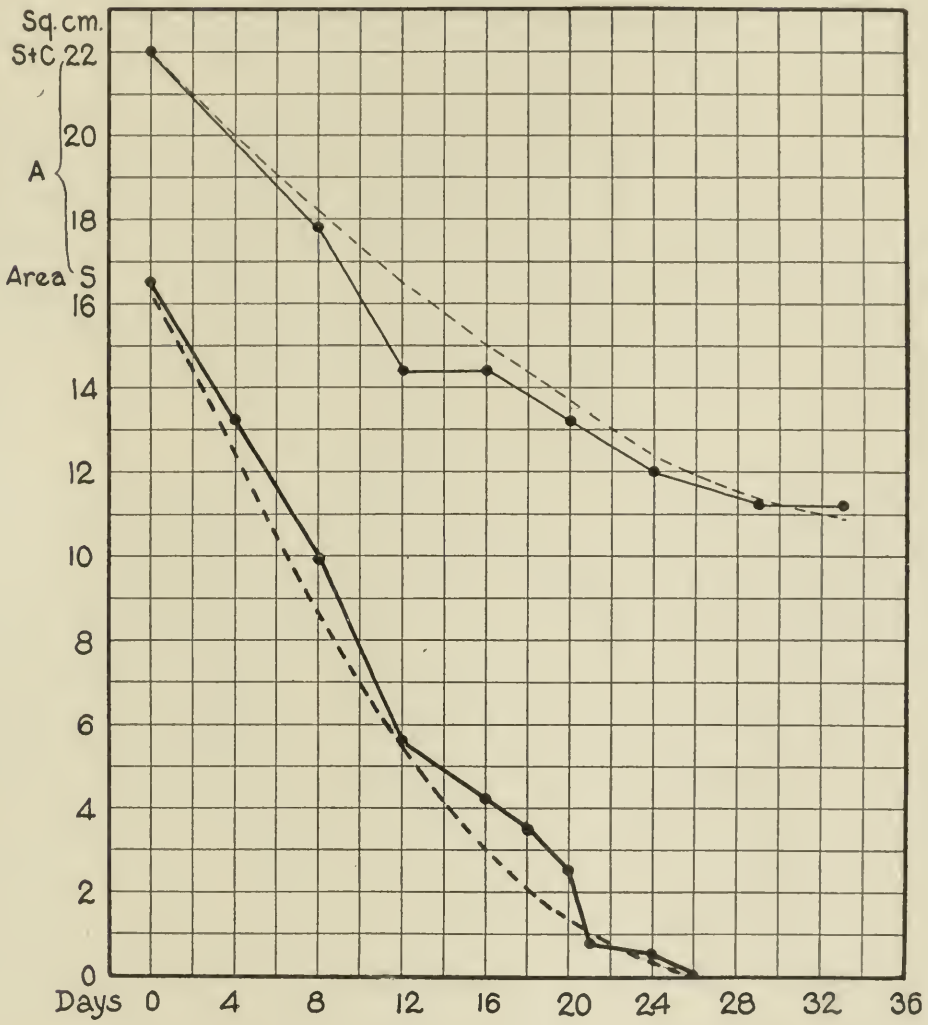
The heavy line represents the decrease of the area of the wound (curve of cicatrization), and the dotted line, the curve calculated according to the equation

$$S_T = S_{0^c} - K\left(T + \frac{T^2}{2p}\right)$$

The decrease in the rate on February 18 is due to infection.



that this factor represents the epithelization. It must not be inferred that the latter remains proportional to $\frac{T^2}{2p}$ and increases at first slowly, then rapidly. On the contrary, we know that epitheliza-




TEXT-FIG. 5. The upper curves are the contraction curves. The dotted curve is calculated according to the formula

$$S_T = S_o e^{-KT}$$

The lower curves are the so called cicatrization curves expressing the decrease of the area of the granulations.

tion, or growth of cells, is likely to be much more active at the beginning of the cicatrization, according to the length of the epithelial edge, and then must decrease in absolute value. In proportion as the

wound decreases, the length of the epithelial edge diminishes, and at the same time the number which measures, in absolute value, the proliferation of cells. But, as it is likely that the number of cells produced by a unit of length is the same for each unit of time, and as, on the other hand, ordinarily the area decreases much faster than the perimeter (four times more rapidly for the square), it is clear that the production of cells by the edges seems to increase and that if it is expressed by units of covered area it increases really with reference to the area of the wound. What must therefore be understood by "the factor represents the epithelization," is that in the considered equation owing to the introduction of this factor, the relations existing between epithelization and the decrease of the area are satisfactorily expressed, and that it enables us to express the result of the phenomena in a way which is in accordance with the facts.

*End of the Phenomenon.*

Since in a logarithmic curve the diminution of the ordinate is always proportional to the ordinate, it never becomes zero. The curve, as well as that which had been established previously, is asymptotic to the axis of the time. But we have already stated, in a former paper, the moment at which cicatrization comes practically to an end.[4] This happens when our methods of measuring are unable to estimate the progress of the phenomenon. This moment is rapidly followed—in a few hours—by complete healing of the wound. The curve practically comes to an end, and experience has shown that it can be arbitrarily stopped, when the ordinate is inferior to 0.4 sq. cm. This means that, when the calculation comes to a figure smaller than 0.4 sq. cm., the corresponding abscissa, that is the time, indicates the date of complete cicatrization. Besides, this conforms to the facts in the majority of cases, as has been shown before, and the errors are small. In all natural phenomena, the law of which is expressed by an exponential equation, the same holds true.

*Numerical Value of the Coefficients. Relation of K, the Index of Cicatrization i, and the Parameter 2p.*

Calculation of fifteen cicatrization curves has shown principally three facts. The first, to which I referred above (page 334), is the pro-

[4] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451; 1917, xxv, 721.

portional variations of $K$ and of the index of cicatrization. Table II shows this plainly. The ratio $\frac{i}{K}$ varies between 1.6 and 1.2 inversely to $i$ and $K$. The second fact is the remarkable constancy of the factor $2p$, or parameter of the parabolæ expressing the acceleration due to the epithelization. The third fact is the relation which seems

TABLE II.

*Comparative Numerical Results.*

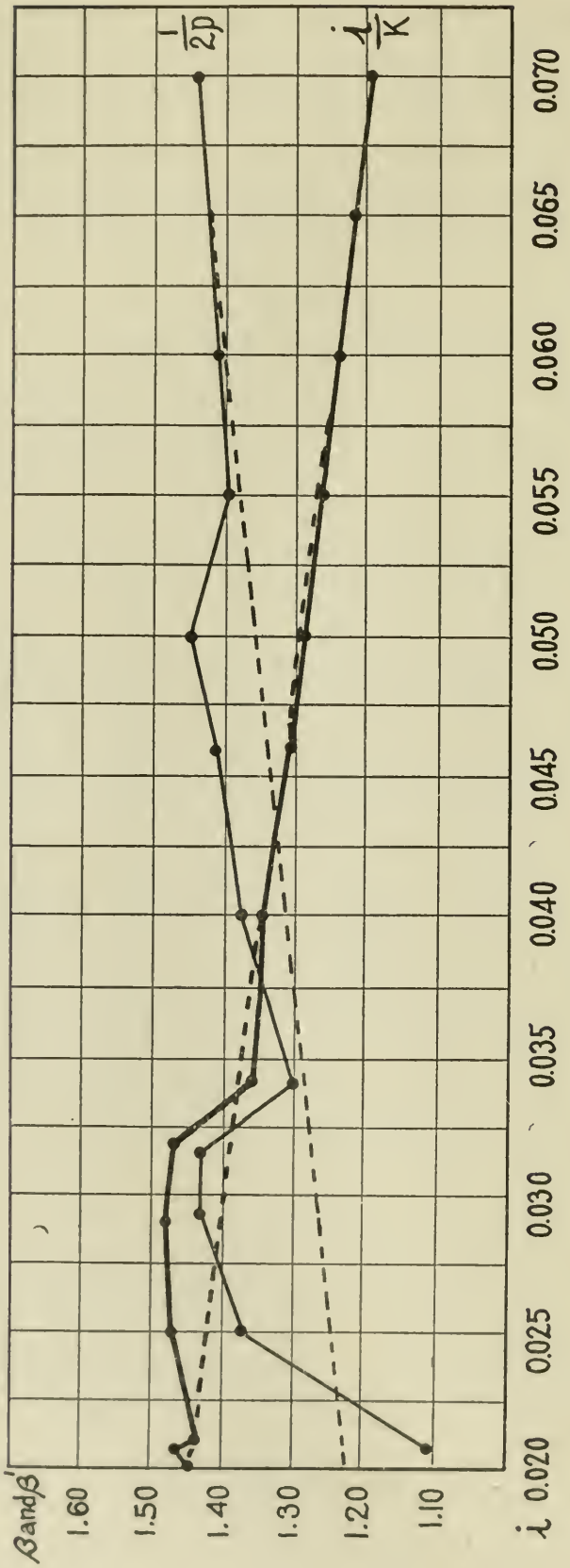
| No. of patient. | Area. | Index $i$. | $K$ | $2p$ | $100\frac{K}{i} = \delta$ | | $\frac{i}{K} = \beta$ |
|---|---|---|---|---|---|---|---|
| | | | | | | $\frac{1}{\beta'}$ | |
| | *sq. cm.* | | | | | | |
| 318 | 64.0 | 0.0200 | 0.0132 | 80 | 66 | | 1.51 |
| 737 | 50.3 | 0.0200 | 0.0138 | 90 | 69 | 81.0 | 1.45 |
| 263 | 61.8 | 0.0205 | 0.0140 | 85 | 68 | 80.5 | 1.46 |
| 360 | 113.0 | 0.0210 | 0.0147 | 90 | 70 | 80.0 | 1.43 |
| 795 | 21.6 | 0.0255 | 0.0174 | 73 | 68.5 | 79.0 | 1.46 |
| 721 | 40.4 | 0.0285 | 0.0192 | 70 | 68 | 78.5 | 1.46 |
| 706 | 27.4 | 0.0325 | 0.0222 | 70 | 68.5 | 77.5 | 1.46 |
| 724 | 13.9 | 0.0346 | 0.0255 | 77 | 74 | 77.0 | 1.36 |
| 725 | 30.6 | 0.0375 | 0.0277 | 75 | 74 | 76.0 | 1.35 |
| 791 | 23.0 | 0.0400 | 0.0295 | 73 | 74 | 75.5 | 1.35 |
| 692 | 31.2 | 0.0420 | 0.0315 | 75 | 75 | 75.0 | 1.33 |
| 722 | 19.0 | 0.0465 | 0.0355 | 71 | 76 | 74.5 | 1.31 |
| 383 | 17.5 | 0.0500 | 0.0387 | 69 | 77 | 74.0 | 1.29 |
| 796 | 8.9 | 0.0550 | 0.0436 | 72 | 80 | 72.0 | 1.26 |
| 715 | 9.5 | 0.0700 | 0.0595 | 65 | 85 | 66.0 | 1.20 |

to exist between $K$, $i$, and $2p$. This is clearly shown by the seventh column in which is reported the term

$$\delta = 100\,\frac{K}{i}$$

The value of $\delta$ for each wound is near enough to $2p$ to allow its substitution for $2p$, approximately. This result is a natural conclusion of the first two remarks, since, if we call $\beta$ the ratio $\frac{i}{K}$, we can write

$$2p = \frac{1}{\beta} = \frac{\delta}{100}$$




TEXT-FIG. 6. Relations between $i$, $K$, and $2p$. The ordinates represent $\frac{i}{K} = \beta$ and $\frac{1}{2p} = \beta'$. Light lines, $\beta'$. Heavy lines, $\beta$.

But this is of immediate value for calculating the curve by equation (9), because, if the coefficient $K$ can be determined by giving two experimental dates 4 days apart, the same process cannot be used for determining the parameter $2p$, for the parabola

$$\alpha = \frac{T^2}{2p}$$

can only be determined when $T$ is great enough; *viz.*, 12 or 16 days. Otherwise the ordinates $\alpha$ are smaller than 1 and the curve is not accurately defined. It is therefore worth while to be able to make an approximate calculation first. If this shows a noticeable error, it is easy to make a correction, as soon as several days have passed. Consequently, it is clear from the above paragraphs that it is possible to calculate the curve resulting from the equation

$$S_T = S_o e^{-K\left(T+\frac{T^2}{2p}\right)} \tag{9}$$

by simply starting from a single measure of the wound and the age of the patient, that is from the index, since

$$K = \frac{i}{\beta}$$

Text-fig. 6 shows the relations between $K$ and $i$. In order to make it clearer, I have plotted close to each point on the observed curve (dotted line) the inverse value of $\beta$; that is, $\frac{1}{\beta}$. The light curve which expresses the observed variations of $\frac{1}{2p} = \beta'$ shows that $2p$ varies approximately inversely to $\beta$ since every point on the curves can be expressed by the inverse value of the ordinate. This means that $2p$ varies inversely to $\delta$. If we admit as a possibility that the light dotted line corresponds to the average mean value of $2p$ and that the values which deviate from it are due to errors of calculation, the probable values of $2p$ can be computed for a certain value of $i$. The figures in the column marked $\frac{1}{\beta'}$ (Table II) may be used for the first approximation of $2p$.

*Calculation of the Coefficients.*

These analogies may be of use in determining $2p$, but sometimes a less accurate approximation is obtained when both $K$ and $2p$ are inferred from the index. In such a case the direct calculation of $K$, which is extremely simple, is of more value. It is deduced, as stated above, from equation (9) which gives

$$K = \frac{1}{T} \operatorname{Log} \frac{S_o}{S_t}$$

$S_o$ being the first measure of the area of the wound, $S_t$ the area at the time $t$ (practically 4 days). After $K$ has been determined, at least two values of $\alpha$ must be calculated unless the relations between the coefficients, mentioned above, are employed. We have stated

$$\alpha = \frac{1}{K} \operatorname{Log} \frac{S_o}{S_T} - T \tag{7}$$

The values of $S$ corresponding to 12 and 20 days, for instance, are taken. (The greater $T$ is, the more accurate the values of $2p$ will be within the limits of 20 to 40 days.) $2p$ is immediately obtained by means of the formula

$$2p = \frac{T^2}{\alpha} \tag{8}$$

Since we have two values of $\alpha$, we obtained two values of $2p$ and the mean value is taken.

The coefficient $K$ is smaller than $i$ and the quantity $\alpha$ must remain positive. If the contrary happens ($\alpha < 0$), a determination of $K$ for a longer period of time (5, 6, or 8 days) must be made. This rarely happens.

*Use of the Equation. Calculation of the Curves; Numerical Examples.*

In order to enable the reader who is unfamiliar with the use of mathematical formulas, to use this equation, we shall make the complete calculation of one curve by using successively the direct calculation, or ordinary method, $i$ being the supposed unknown, and then the method based upon the analogies existing between the coefficients. The difference in accuracy of both methods will thus be noted. Whenever the index $i$ varies around 0.04 the results obtained by the second

technique are excellent. The reason is evident from Text-fig. 6 in which the values of the coefficients are in accordance with this particular value of $i$.

(*1*) *Direct Calculation.*—Only the initial area and that after 4, 8, or 20 days are given (Table III).

TABLE III.

*Example of Direct Calculation.*

| $T$ | $S$, observed area. | Log $S$ | Log $\frac{S_o}{S}$ | $\alpha$ | $2p$ | Calculated area. |
|---|---|---|---|---|---|---|
| | *sq. cm.* | | | | | *sq. cm.* |
| 0 | 40.4 | 1.602 | | | | |
| 4 | 33.5 | 1.525 | 0.077 | 0 | | |
| 8 | 27.0 | 1.431 | 0.171 | 0.9 | 71 | 27.0 |
| 20 | 12.5 | 1.097 | 0.505 | 6.2 | 65 | 12.8 |
| 28 | 6.8 | | | | | 7.0 |
| 36 | 3.5 | | | | | 3.5 |
| 44 | 1.7 | | | | | 1.7 |
| 54 | 0.6 | | | | | 0.6 |

The calculation requires accordingly (*a*) the determination of $K$

$$\left(K = \frac{\text{Log } S_o - \text{Log } S_T}{T}\right), \qquad K = \frac{0.077}{4} = 0.0192$$

(*b*) the determination of $\alpha$ (8th day)

$$\left(\alpha = \frac{\text{Log } S_o - \text{Log } S_T}{K} - T\right), \qquad \alpha = \frac{0.171}{0.0192} - 8 = 0.9$$

(*c*) the determination of $2p$ (8th and 20th days)

$$\left(2p = \frac{T^2}{\alpha}\right), \qquad 2p = \frac{64}{0.9} = 71$$

and finally the calculation of the points of the curve for the given times by formula (9)

$$\text{Log } S_T = \text{Log } S_o - K\left(T - \frac{T^2}{2p}\right)$$

(*2*) *Indirect Calculation.*—This is based only upon the relations previously stated between $i$, $K$, and $2p$. $2p$ may be taken either from

Table II or from Text-fig. 6, for the given value of $i$. In the preceding example $i = 0.0285$ (Table V). For this value, the table indicates $\frac{1}{\beta'} = 2p = 78.5$. The factor given by Text-fig. 6 is 1.41. Hence $K = \frac{i}{1.41} = 0.0202$. By applying the formula the areas given in Table IV are calculated (compare with Table III).

This shows that the values are as good as those obtained by the direct method, sometimes even better, because in the latter method $2p$ has only been determined from two points on the curve, which is a cause of error.

A direct determination of $K$ can also be made and only the value of $2p$ read in the table. The results obtained by this intermediate technique are good, but it shows no particular advantage, and, on the contrary, introduces a new factor of error and requires more time.

TABLE IV.

| Area ($S$). | | | | | |
|---|---|---|---|---|---|
| 8th day. | 20th day. | 28th day. | 36th day. | 44th day. | 54th day. |
| *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| 27.0 | 12.4 | 6.8 | 3.5 | 1.6 | 0.5 |

It must be borne in mind that the determination of a curve by a single measure of the area and the normal index presents many advantages which may be of greater interest than the perfect coincidence between two curves obtained by two equations of different form. The advantages are: (1) the possession of the normal curve of cicatrization corresponding to the normal index, characterizing the age of the patient; this curve is used as a standard with which the individual curve is compared, if they do not agree; (2) the elimination of errors due to two measures of the wound, because during the time elapsed between the measures—4 days for example—a slight acceleration or a slight lessening in the rate might have occurred. The so called indirect method, therefore, should be used when the normal curve of a wound is to be calculated. In order to facilitate this calculation, I have drawn Text-fig. 7 similar to Text-fig. 6, except that the ob-

TABLE V.

*Calculation of the Curve of Cicatrization.*

*The Two Coefficients of the Formula*

$$S' = S\,[1-i\,(t+\sqrt{nt})].$$

| Area of wound. | 1st coefficient—index of cicatrization $i$. Age of patient. | | | | | 2nd coefficient (time coefficient) $t+\sqrt{nt}$. |
|---|---|---|---|---|---|---|
| | 20 yrs. | 25 yrs. | 30 yrs. | 32 yrs. | 40 yrs. | |
| *sq. cm.* | | | | | | |
| 150 and over. | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 6.00 |
| | | | | | | 6.81 |
| | | | | | | 7.43 |
| 140 | 0.0210 | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 8.00 |
| | | | | | | 8.45 |
| 130 | 0.0220 | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 8.90 |
| | | | | | | 9.30 |
| 120 | 0.0225 | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 9.65 |
| | | | | | | 10.00 |
| 110 | 0.0240 | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 10.32 |
| | | | | | | 10.64 |
| 100 | 0.0250 | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 10.93 |
| | | | | | | 11.21 |
| 90 | 0.0275 | 0.0220 | 0.0200 | 0.0200 | 0.0200 | 11.48 |
| | | | | | | 11.75 |
| 80 | 0.0300 | 0.0230 | 0.0200 | 0.0200 | 0.0200 | 12.00 |
| | | | | | | 12.25 |
| 70 | 0.0325 | 0.0250 | 0.0200 | 0.0200 | 0.0200 | 12.48 |
| | | | | | | 12.72 |
| 60 | 0.0355 | 0.0300 | 0.0225 | 0.0200 | 0.0200 | 12.95 |
| | | | | | | 13.16 |
| 50 | 0.0400 | 0.0340 | 0.0265 | 0.0230 | 0.0200 | 13.37 |
| | | | | | | 13.60 |
| 40 | 0.0445 | 0.0400 | 0.0310 | 0.0270 | 0.0220 | |
| 30 | 0.0500 | 0.0450 | 0.0375 | 0.0330 | 0.0260 | |
| 25 | 0.0540 | 0.0500 | 0.0400 | 0.0375 | 0.0290 | |
| 20 | 0.0580 | 0.0540 | 0.0465 | 0.0425 | 0.0325 | |
| 15 | 0.0645 | 0.0600 | 0.0525 | 0.0475 | 0.0380 | |
| 10 | 0.0700 | 0.0660 | 0.0625 | 0.0550 | 0.0450 | |
| 5 and under. | 0.0800 | 0.0750 | 0.0700 | 0.0700 | 0.0700 | |

served points are suppressed and the scale of the ordinates is larger, so that a greater accuracy may be obtained. To show the degree of approximation obtained by the new technique I have collected the calculations of four wounds. The figures calculated according to the extrapolation and exponential equations correspond to every observed area. These curves have been chosen intentionally, so that their indices are different. The calculation of the coefficients $K$ and $2p$ is then simply done by looking for the index in Table V in function of the age of the man and of the area of the wound; then by using the relations (Table II)

$$K = \frac{i}{\beta} \text{ and } 2p = \frac{100}{\beta'}$$

calling $\beta$ the observed values of $\frac{i}{K}$ (solid line) and $\beta'$ the observed values of $\frac{1}{2p}$ (dotted and broken line), the values of $2p$ can also be found in Text-fig. 7, since the values of Table II have been calculated from this straight line. Text-fig. 7 is only used in order to give two conversion factors $\beta$ and $\beta'$, to be applied for computing $K$ and $2p$.

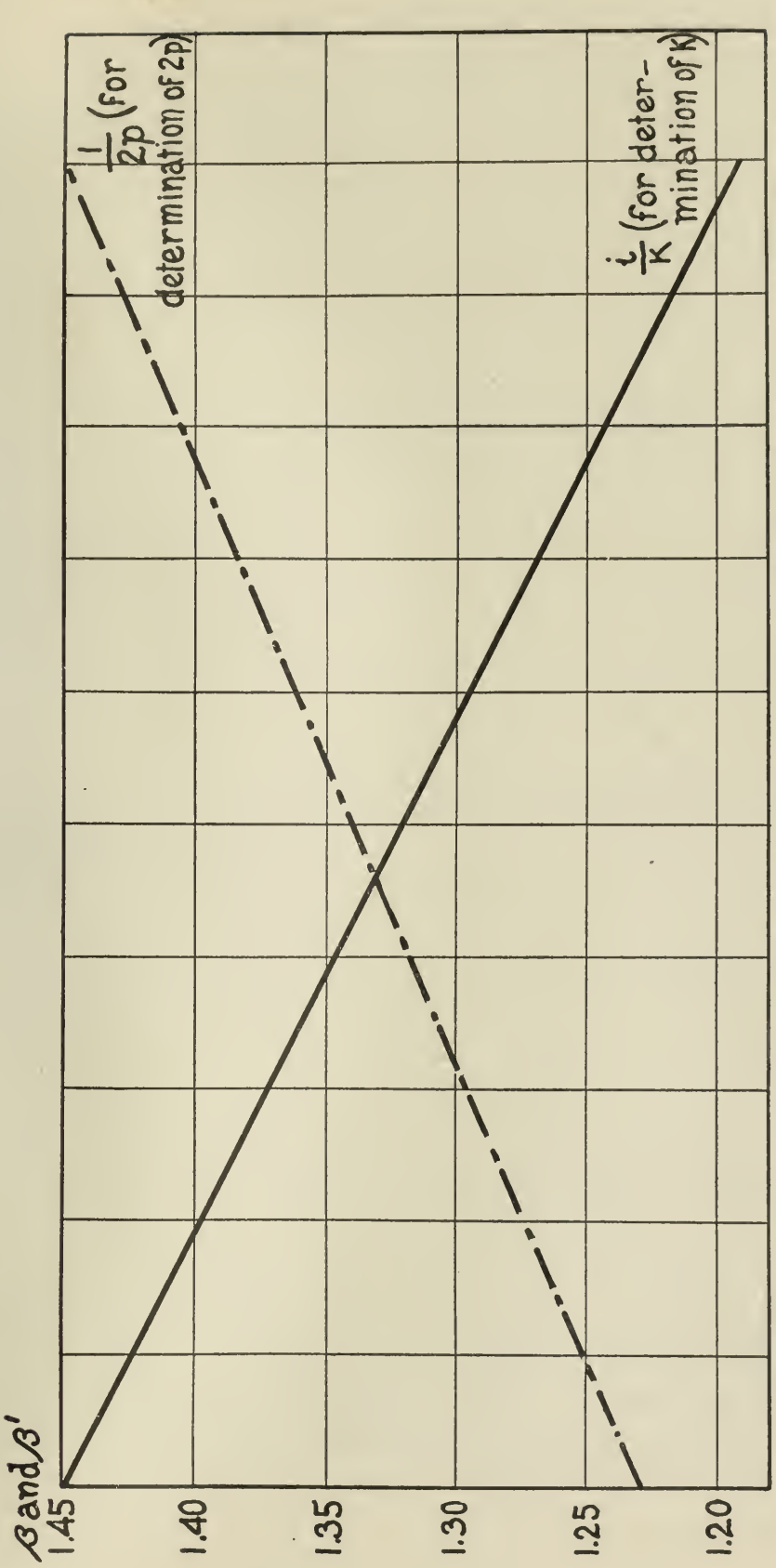
### *Comparative Examples.*

#### *Patient 360.*

$$S_o = 129.4 \text{ sq. cm.}, i = 0.021, \beta = 1.44, \beta' = 1.23, K = \frac{i}{\beta} = 0.01458,$$

$$2p = \frac{1}{\beta'} = \frac{1}{1.23} \times 100 = 81.$$

| Area. | | 8th day. | 20th day. | 44th day. | 60th day. | 76th day. | 84th day. |
|---|---|---|---|---|---|---|---|
| | | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| Observed | | 105.0 | 57.0 | 13.8 | 4.1 | 1.8 | 0.6 |
| Calculated | Equation (1) | 96.8 | 55.9 | 14.0 | 4.7 | 1.9 | 1.0 |
| | " (9) | 96.5 | 56.0 | 13.4 | 3.9 | 1.4 | 0.4 |




TEXT-FIG. 7. Relations between $i$, $K$, and $2p$. The chart gives the ratio $\beta = \frac{i}{K}$ and $\frac{1}{2p} = \beta'$, by means of which $K$ and $2p$ can be calculated.

*Patient 488.*

$S_o$ = 34.5 sq. cm., $i$ = 0.03, $\beta$ = 1.40, $\beta'$ = 1.27, $K$ = 0.0214, $2p$ = 79.

| Area. | 8th day. | 20th day. | 32nd day. | 44th day. | 48th day. |
|---|---|---|---|---|---|
| | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| Observed | 20.6 | 11.0 | 4.0 | 1.7 | 0.9 |
| Calculated { Equation (1) | 22.4 | 9.9 | 3.7 | 1.3 | 0.8 |
| Calculated { " (9) | 22.2 | 10.0 | 3.8 | 1.2 | 0.7 |

*Patient 694.*

$S_o$ = 44.3 sq. cm., $i$ = 0.0425, $\beta$ = 1.33, $\beta'$ = 1.33.

| Area. | 8th day. | 16th day. | 20th day. | 28th day. |
|---|---|---|---|---|
| | *s .* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| Observed | 23.6 | 11.2 | 8.5 | 2.5 |
| Calculated { Equation (1) | 23.5 | 10.5 | 6.9 | 2.4 |
| Calculated { " (9) | 23.1 | 10.7 | 6.8 | 2.6 |

*Patient 519.*

$S_o$ = 19.0 sq. cm., $i$ = 0.0570, $\beta$ = 1.26, $\beta'$ = 1.39.

| Area. | 4th day. | 12th day. | 20th day. | 28th day. |
|---|---|---|---|---|
| | *sq. cm.* | *sq. cm.* | *sq. cm.* | *sq. cm.* |
| Observed | 12.2 | 4.2 | 1.0 | 0.4 |
| Calculated { Equation (1) | 12.5 | 4.4 | 1.2 | 0.3 |
| Calculated { " (9) | 12.2 | 4.4 | 1.3 | 0.3 |

## CONCLUSION.

1. The law of cicatrization of surface wounds may be expressed by an exponential formula in which the two coefficients may be determined.

2. A simple relation exists between these coefficients and the index, $i$, of cicatrization, previously established in function of the age of the patient and of the area of the wound.

3. The proposed equation with a simplified exponent, reduced to a single coefficient, expresses satisfactorily the phenomenon of contraction.

# OBSERVATIONS ON THE WASSERMANN REACTION.

## A Comparison of the New System of Noguchi with That Using Cholesterolized Antigen According to McIntosh and Fildes.

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(Received for publication, January 23, 1919.)

Among the many modifications of the Wassermann reaction, that of Noguchi,[1] introduced a number of years ago, in which use is made of the acetone-insoluble lipoids extracted from certain tissues and the anti-human hemolytic system, is doubtless the most promising from a theoretical standpoint. In practice we had found it a troublesome system to apply particularly because the collection of the human cells for the immunization of rabbits and carrying out the tests is laborious and time-consuming, and also because the production of the anti-human amboceptor in the rabbit is less satisfactory than that of anti-sheep amboceptor. We had come to the conclusion that if a certain allowance was made for reactive anti-sheep amboceptor, as can be done by an intermediate incubation period of 15 to 20 minutes after adding the sheep cells, but before adding the anti-sheep amboceptor, the results did not differ materially from the human hemolytic system. For a long time we used the Noguchi antigen with satisfaction.

McIntosh and Fildes[2] presented an application of a principle introduced by Sachs (the addition of cholesterol to alcoholic tissue extracts), and Richardson[3] compared the reactions with this "choles-

[1] Noguchi, H., Serum diagnosis of syphilis, Philadelphia, 1910.

[2] McIntosh, J., and Fildes, P., An investigation of the value of certain antigens for use in the Wassermann reaction, in particular of Sach's new antigen, *Z. Chemotherap., Orig.*, 1912–13, i, 79.

[3] Unpublished observations at the Pennsylvania Hospital.

terolized antigen" with those obtained with the Noguchi antigen with the anti-sheep hemolytic system. He studied about 2,000 reactions, and, like other investigators, found that the cholesterolized antigen gave the higher percentage of positive reactions, but that in most cases this was justified by the facts. Eight cases were found in which there was no evidence of syphilis other than the positive reaction, but there was no evidence to the contrary and it was considered that the result was on the whole satisfactory. This antigen is the easiest to obtain in quantity and is of relatively constant quality as compared with others. It was therefore adopted and has been used continuously for a number of years in these laboratories. More recently the carrying out of the reactions has passed into other hands, and we have had some reason to question the accuracy of the opinion previously held as to the safety of the cholesterolized antigen from the diagnostic point of view.

In the meantime, opinion elsewhere seems to be divided. Some competent observers have found the cholesterolized preparation satisfactory. In the hands of others it seems to be too sensitive; that is, it gives rise to false positives.

Recently Noguchi[4,5] has proposed the use of the native human complement in the reaction with his acetone-insoluble lipoids as antigen. He states that with the quantities he specifies satisfactory results can be obtained, fully comparable with those derived from the use of his older system. This system makes unnecessary the continued collection of guinea pig serum, and this may be expected to compensate for the trouble in regard to human red cells. It is, of course, of importance at times to be able to carry out the method when guinea pig serum may not be available.

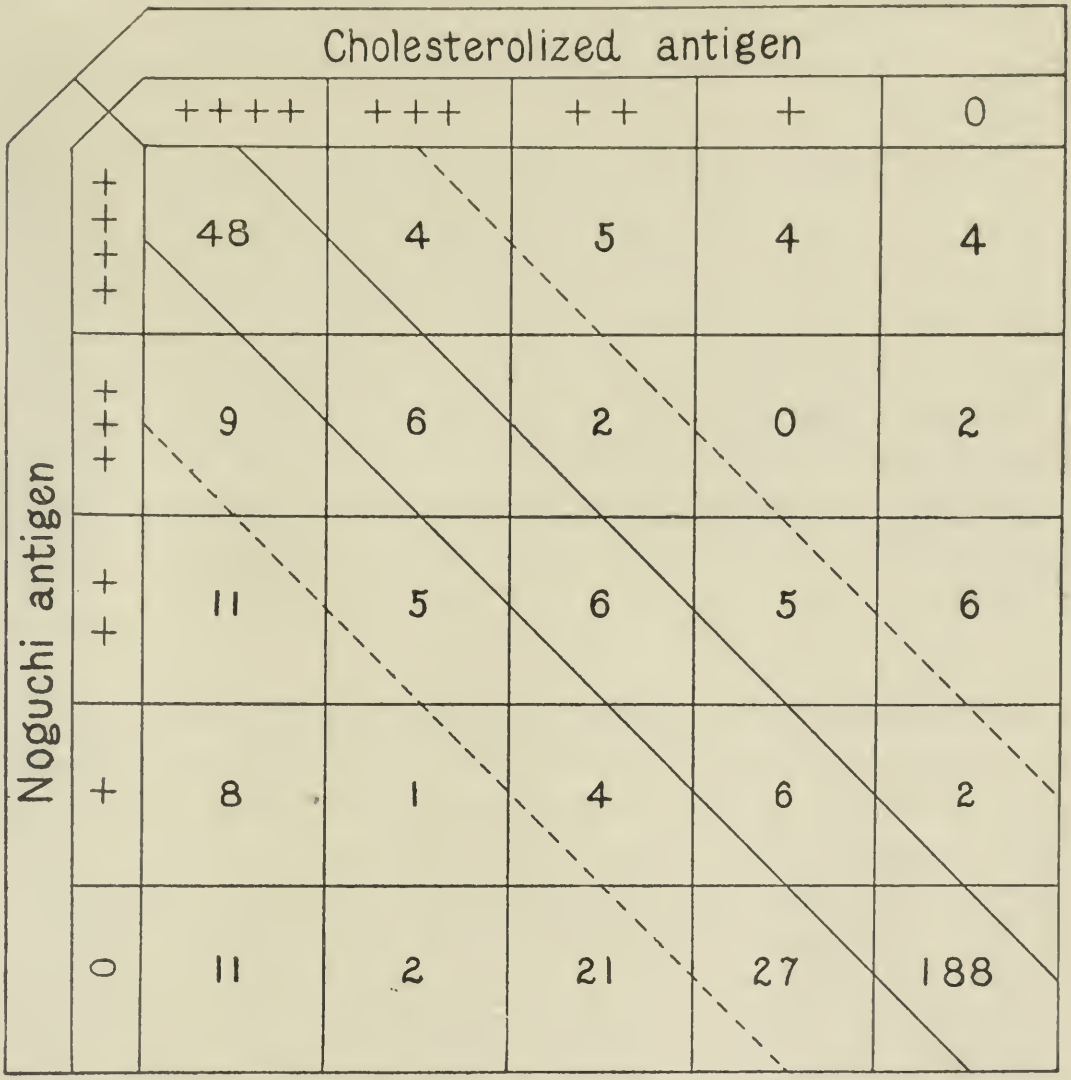
We have attempted, therefore, to review the question of the Wassermann reaction as practically applied with the purposes of (1) reconsidering the suitability of the cholesterolized antigen, and (2) examining the merits of the newer Noguchi system.

[4] Noguchi, H., A method of facilitating the serum diagnosis of syphilis under war conditions, *J. Am. Med. Assn.*, 1918, lxx, 1157.

[5] Noguchi, H., A homohemolytic system for the serum diagnosis of syphilis, *J. Exp. Med.*, 1918, xxviii, 43.

RESULTS.

It has been assumed on the basis of the author's report that the two systems of Noguchi would have equal value and that comparison of one of them with the results by cholesterolized antigen should




TEXT-FIG. 1. The figures in the chart indicate the number of sera giving deviations as indicated with both the cholesterolized antigen and according to Noguchi's homohemolytic system. The total comprises 387 different sera.

establish the place of the latter. We have therefore done our reactions comparing the newer Noguchi system with the system which makes use of cholesterolized antigen and the anti-sheep hemolytic reagents. The general results are displayed in Text-fig. 1.

It is apparent that in a large majority of the cases the two methods of work have an equal value (central diagonal space) or one essentially equal (adjacent diagonal spaces). It is also clear that the numbers toward the lower left corner (cholesterol reaction positive, Noguchi reaction negative) considerably outnumber those toward the upper right (cholesterol reaction negative, Noguchi reaction positive). In other words, the cholesterolized antigen is considerably more reactive.

Which method develops the facts most nearly in accord with the truth as to the existence of syphilis in the particular cases? Of the ten cases in which the Noguchi system gave a positive and the other a negative or weak reaction, eight were unquestionably syphilitic. One of the others was possibly syphilitic. In the tenth case, that of an apparently healthy young man with a fractured jaw, no decisive evidence could be obtained. In all these cases except one, who was an undoubted syphilitic, doubling the amount of serum used (making 0.2 cc.) in the reaction with the cholesterolized antigen perceptibly increased the intensity and in most instances rendered it entirely positive.

*Material.*

Before taking up the question of whether a positive reaction with the cholesterolized antigen means syphilis, even though the Noguchi reaction be weak or absent, it is perhaps best to consider the material which we have had for study. The majority of our reactions were carried out on known cases of syphilis, who were under treatment. In most of these the evidence of disease was clear apart from the Wassermann reaction. Most of the cases which showed a positive cholesterol and negative Noguchi reaction come in this group. As controls we examined the blood of twenty-nine persons in good health who were sufficiently known to us to make it appear improbable that they were suffering from syphilis. One of these gave +++ reaction with cholesterolized antigen and on the same occasion gave a ++ Noguchi reaction. On another occasion the same person gave a ++ cholesterol and a negative Noguchi. Two others in the group gave a ++ cholesterol reaction and a negative Noguchi. Further, the blood of 109 patients at one time available in the hos-

pital was examined without selection. Whether by chance or not these results, which will be considered in detail later, do not bear on the present point as the two reactions were practically alike in all instances.

Of the cases in which the cholesterol reaction was positive (++++ or +++), the Noguchi reaction being at the same time weak or negative, five could not be satisfactorily determined although there is no reason to believe that the reaction is in error. The remainder were definitely syphilitic and there is every reason to believe that the reaction with cholesterolized antigen is a better expression of their condition than that done by the Noguchi method.

There are twenty-one reactions showing a ++ cholesterol with a negative Noguchi reaction. In this series these reactions are for the most part the evidence of a fading out of the activity of the serum in the course of specific treatment. In view of the three reactions of similar intensity among twenty-nine probably normal individuals such degrees of activity cannot, of course, be held to be of any decisive value from a diagnostic view-point. Whether the six instances in which the Noguchi reaction was ++ and the cholesterol reaction negative are similarly lacking in diagnostic significance may be open to question, but we are inclined to the belief that they are, except, as may sometimes happen, when doubling the amount of test serum leads to a stronger reaction with cholesterol.

*Interpretation of Results.*

In view of the results in the unselected series of patients in the hospital, it appears to be useless to discuss the question of just which intensity of reaction, with either antigen or both, justified a diagnosis of syphilis. Among these 109 persons there were several cases in whom the diagnosis of syphilis was presumptive on other grounds. These gave clear-cut reactions with both systems. There were two patients who gave ++++ Noguchi reactions with weak cholesterol reactions. The status of these cases remains in doubt. In addition, the cases shown in Table I gave more or less reaction.

These cases comprise about half the febrile cases in the hospital at the time. The others, rheumatic fever, endocarditis, and lobar pneumonia, as well as about 50 cases in the surgical wards, gave

negative reactions. It is evident, therefore, that the Wassermann reaction must be used with some caution and that it must always be valued in conjunction with a presumptive diagnosis based on other grounds. With these facts in mind it may be fairly stated that from the point of view of establishing a diagnosis the two forms of reaction have approximately equal value. The cholesterol reaction is somewhat more sensitive and the weaker grades are to be viewed with a little less confidence perhaps than similar weak reactions with the Noguchi system. Whenever possible there would

TABLE I.

| Case No. | Diagnosis. | Cholesterol reaction. | Noguchi reaction. |
|---|---|---|---|
| 13 | Typhoid fever | +++ | ++++ |
| 16 | " " | ++ | 0 |
| 18 | " " | ++++ | +++ |
| 20 | Heat stroke | + | 0 |
| 24 | Typhoid fever | ++++ | ++ |
| 25 | " " | ++++ | ++ |
| 26 | Heat stroke | ++++ | ++++ |
| 28 | Typhoid fever | + | 0 |
| 29 | Heat stroke | + | 0 |
| 30 | Lobar pneumonia | +++ | + |
| 45 | Gastroenteritis | 0 | ++ |
| 54 | Asthma | 0 | ++ |
| 57 | Tuberculosis; peritonitis | ++ | 0 |
| 58 | Acute enteritis | 0 | ++ |

appear to be a real advantage in viewing the two systems side by side, holding for particularly careful scrutiny cases in which the results do not check.

Fully as important as its application to diagnosis is the use of the Wassermann reaction as a control for treatment. Here any difference that may exist in sensitivity is in favor of the continued use of the cholesterolized antigen. It may also be profitable to use the two systems side by side. One purpose of this would again be that of having a precautionary check. There is imperfect evidence in our hands, however, that under treatment the two systems may vary in some degree independently of one another, and it is possible that useful information may be obtained by following out this suggestion.

Up to this point we have spoken of these two types of reaction as though the results with either were always unmistakable and arrived at without difficulty. This is by no means the case. Exception has frequently been taken to the use of cholesterolized antigen because of irregularities which develop with successive reactions with the same samples of serum. In order to make the reaction safe it seems to us necessary to use a slight excess over the amount of complement usually advised for Wassermann work. Our custom is to use $2\frac{1}{2}$ units of both complement and amboceptor. The final antigen dilution should be used within a few minutes after it is made and should be made in accordance with McIntosh and Fildes' directions; that is, the mixture with salt solution should be rapidly accomplished. We put the salt solution (24 cc.) in an Erlenmeyer flask of 50 cc. capacity, float the alcoholic solution very carefully on the surface, and then mix the two with one quick vigorous shake. The titration of reagents is carried out completely each time on the basis of $\frac{1}{2}$ hour incubation in the water bath and the incubation after the addition of the hemolytic system is likewise $\frac{1}{2}$ hour.

Neither is the Noguchi system in its present form free from difficulty. It should be noted that in the two publications on the subject Noguchi has recommended the use of two different quantities of human serum. He first suggested 0.1 cc. as the proper quantity, later changing this to 0.2 cc. in his final paper. Neither recommendation appears to us to be adequate. As a matter of actual observation there are great differences in the susceptibility of various samples of human red corpuscles to hemolysis by human complement and rabbit anti-human amboceptor. These differences are quantitatively such that a given serum may present insufficient complement for the reaction or may have more than four units. From 70 consecutive tests we found that with susceptible corpuscles every fresh human serum has complement sufficient for the test. Certain sera with these corpuscles may contain as much as eight units of complement. With resistant corpuscles no serum may have more than two units and one in ten may show no complement whatever. An intermediate condition is more common and was evidently encountered by Noguchi. If exact work is to be done account must be taken of these facts.

We have found it best to conduct each test with three quantities of serum, 0.2, 0.1, and 0.05 cc., each quantity having its antigen and control tubes. The result is then scarcely ever in doubt. It would doubtless be still better to standardize the reaction to a certain extent by selecting a single suitable person, preferably one with susceptible corpuscles, as a source of the red cells.[6] If this were done it would probably be safe to rely on two of the above quantities of serum, either the greater or the lesser pair as the susceptibility of the cells indicated.

Another objection to the system which at first sight seems important is negligible in practice. It is obviously impossible to control the anticomplementary value of the antigen for each separate complement where the complement is furnished by the test serum. The antigen which we have been using has had an apparent anticomplementary value of about ++ on the unit value of complement and this can be allowed throughout the tests whenever deviation occurs. For each lot of antigen this would necessarily be determined by comparative observation on a number of negative sera. Noguchi speaks of his antigen's being entirely without anticomplementary value in the quantity recommended. In this particular our application of the method can doubtless be somewhat improved upon.

## SUMMARY.

The Wassermann reaction carried out according to the method of McIntosh and Fildes, with cholesterolized antigen and with certain allowances for the presence of native anti-sheep amboceptor, leads to about the same result as when it is done according to the recent proposal of Noguchi, with the native human complement and acetone-insoluble lipoids as antigen. The differences are such as to suggest that from the point of view of diagnosis the Noguchi method is the more conservative but that there is definite advan-

[6] Observations which have been begun but are as yet unfinished indicate that the source of variation in the resistance of the corpuscles may be sought rather in the way the cells are kept or their age outside the body than in any true variation from person to person. The outcome of this study in no way influences the practical significance of the considerations put forward above.

tage in using two methods as distinct in origin of materials as these, partly for the purpose of control and partly in the hope of acquiring new information of importance. As a measure of control of treatment the cholesterol antigen appears to us to be the more valuable. The Wassermann reaction alone, by whatever method it may be done, can only be used in the diagnosis of syphilis in conjunction with presumption based on other grounds. That it fails to appear in a considerable percentage of syphilitics is well known. That the reaction is positive in other conditions is not so generally recognized. Fresh instances of this in certain febrile cases are here recorded.

# MIGRATION OF PARASITES AS THE CAUSE OF ANEMIA IN ÆSTIVO-AUTUMNAL MALARIAL INFECTIONS.*

By MARY R. LAWSON, M.D.

(*From the Laboratory of Dr. Mary R. Lawson, New London.*)

Plates 17 to 19.

(Received for publication, December 31, 1918.)

## *Malarial Anemia.*

Malarial infections are characterized by the early development of a more or less severe anemia. As a rule, the æstivo-autumnal infections present the gravest manifestations. The severity of the anemia and its rapid appearance have frequently been noted.

Rossoni[1] states that no acute infection results in so active a deglobulization as does malarial fever, and that in all cases there is an immediate diminution in the number of corpuscles and the amount of hemoglobin. Manson[2] writes: "We find a degree of oligocythæmia greatly in excess of anything we might expect, or which can be accounted for by, or in correspondence with, the proportion of corpuscles attacked and directly consumed by the plasmodium."

## *Loss of Red Corpuscles in the Intervals between the Paroxysms.*

Each parasite destroys several red corpuscles in the course of its development, the result of which is not only the loss of corpuscles with each paroxysm, but a loss in the intervals between paroxysms. That this interval loss has been noted might be understood from the following observations.

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] Rossoni, quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 60.

[2] Manson, P., Tropical diseases, a manual of the diseases of warm climates, London, 3rd edition, 1903, 88.

Thayer and Hewetson[3] state that in pernicious cases the number of red corpuscles may fall between the paroxysms. Marchiafava and Bignami[4] observed that anemia is produced in cases of pernicious fever which are not accompanied by elevations of temperature, the so called larval or masked pernicious fevers. They state: "Thus a patient of vigorous constitution in the first four days of a quotidian fever, or a remittent fever of first invasion, may show a reduction to 2,000,000 red blood corpuscles, or there may even be a reduction of 1,000,000 per cubic millimetre within twenty-four hours." Rossoni[5] states that the loss in hemoglobin and corpuscles is rarely evident during the paroxysm, but begins with apyrexia and may continue for several days afterwards. Dionisi[6] in a case of æstivo-autumnal infection observed a reduction of 500,000 red corpuscles in 12 hours, and he noted a fall from 3,200,000 to 2,300,000 corpuscles during the first 6 days of apyrexia in spite of the use of iron. Kelsch[7] saw a diminution of red corpuscles as low as 500,000 per c.mm. This diminution occurs, according to him, irregularly. Ewing[8] noted that the anemia may progress in the afebrile periods.

I have observed a continued loss of red corpuscles in the intervals between paroxysms.

### *Theories Advanced to Account for an Anemia Out of Proportion to the Number of Parasites Present.*

The belief has been general that the only actual destruction of red corpuscles by parasites occurred when the parasites segmented, the destruction of the corpuscles corresponding to the segmentation of the parasites. Therefore when an anemia occurred out of proportion to the number of parasites present, provided each parasite destroyed but one corpuscle, certain theories were formulated to account for it.

[3] Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 57.

[4] Marchiafava, E., and Bignami, A., in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 187, 188.

[5] Rossoni, quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 61.

[6] Dionisi, quoted from Ewing, J., Clinical pathology of the blood, New York, 1903, 2nd edition, 459, 460.

[7] Kelsch, quoted from Mannaberg, J., The malarial parasites. A description based upon observations made by the author and by other observers, translation by Felkin, R. W., London, 1894, 384.

[8] Ewing, J., *J. Exp. Med.*, 1900-01, v, 460.

Mannaberg[9] states: "It is possible that damage to the non-infested blood corpuscles is due to the parasitic poison which is dissolved in the *liquor sanguinis*. So we see that the red blood corpuscle in malarial patients may be attacked in two ways, but their destruction is far more due to direct invasion by the parasites than to the dissolved poison." Marchiafava and Bignami[10] write: "Nothing, however, forbids a belief that a special toxin of parasitic origin causes destruction of the red corpuscles by a specific action of its own. . . . . although the researches so far pursued with the purpose of directly proving the existence of poisons caused by the parasites have given negative results." Thayer[11] states "that there may well be other substances present in the circulation which result in the destruction of a number of non-parasitiferous elements." Ewing[12] writes: "Many factors indicate that the post critical anæmia is principally referable to globucidal action of the serum dependent upon the presence of a malarial toxin," one of the facts in support of this view being the "disproportion between the anæmia and the number of parasites present." Viola[13] believes that the destruction of red corpuscles depends only in a minor degree upon the direct action of the parasites and to a greater extent upon their toxic products.

As far as I can ascertain there has been no convincing evidence in support of the above views.

*The Hemoglobin in Malarial Infections.*

Reduction of hemoglobin out of proportion to the loss of red corpuscles is frequently observed. This reduction is easily explained. Two facts should make it clear: (1) a total destruction of red corpuscles accompanied by (2) a partial destruction by the parasites. There are at all times a certain number of parasites attached to corpuscles which are partially decolorized by parasitic action. When a red count and hemoglobin estimate are taken, each partially decolorized corpuscle counts as a corpuscle, and there is a partial loss of hemoglobin plus the total loss.

[9] Mannaberg, J., The malarial parasites. A description based upon observations made by the author and by other observers, translation by Felkin, R. W., London, 1894, 384.

[10] Marchiafava, E., and Bignami, A., in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 199.

[11] Thayer, W. S., Lectures on the malarial fevers, New York, 1897, 185.

[12] Ewing, J., Clinical pathology of the blood, New York, 1903, 2nd edition, 461.

[13] Viola, quoted from Marchiafava, E., and Bignami, A., in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 193.

*Migration of Parasites in Æstivo-Autumnal Infections (Figs. 1 to 93).*

It is practically impossible to ascertain the total number of migrations in the æstivo-autumnal infections as so few of the developmental stages are present in the blood. Migration of parasites is undoubtedly going on in the internal organs with consequent loss of red corpuscles. As stated above, each parasite destroys more than one red corpuscle; that is, after destroying one corpuscle, the parasite migrates to another and proceeds to destroy that. In the intervals between attachments the parasite is, for brief periods of time, free in the blood serum. All the parasites of one brood do not migrate at one time any more than do all the parasites of one brood segment at once.

In films containing free parasites in migration (Figs. 1 to 30, 35 to 64, and 66 to 69) one finds, usually, other parasites in similar stages of development, attached to dehemoglobinized red corpuscles (Figs. 65 and 86 to 93) or to corpuscular skeletons (Figs. 31 to 34) as well as attached to red corpuscles whose hemoglobin appears to be intact (Figs. 70 to 84).

The above stages of migration, when seen in one film, indicate that the process of migration is rapid and that the parasites do not long remain free. One would naturally expect an immediate attachment of the parasites when the surroundings are favorable to their growth, and no quinine or other injurious drug is present. I have seen parasites attaching themselves to fresh corpuscles before they had entirely abandoned the dehemoglobinized corpuscles.

Mannaberg [14] observed a tertian parasite (not a flagellating body) abandon a corpuscle, and he states: "It occurs with such rapidity and in such an unexpected manner that the details cannot be appreciated." In 1899, Dock[15] described what I believe to be migrating parasites. In several instances he saw free parasites. He pictures eight changes of form in a small, free, hyaline body, the observation lasting 5 minutes. He states that certain parasites seemed to have an especial tendency to escape from the blood corpuscles at early periods. And he seemed to be in doubt as to whether the free parasites were varieties of

[14] Mannaberg, J., The malarial parasites. A description based upon observations made by the author and by other observers, translation by Felkin, R. W., London, 1894, 344.

[15] Dock, G., *Med. News*, 1890, lvii, 61, 63.

the forms which carry on the typical process in malaria, or whether they had an independent existence. Bignami and Bastianelli[16] believed that, in the presence of a large number of free parasites, they had found evidence of an early destruction by decolorization of a large number of parasitiferous corpuscles. In 1914, Macfie,[17] in a case of æstivo-autumnal infection, observed an undoubted migration. He reports that a considerable number of free parasites were seen, many of which appeared to be young parasites which had not yet attached themselves. Other parasites, he states, were definitely ring-shaped, not infrequently possessing two chromatin masses. Undoubtedly the ring-shaped parasites and the parasites with the two chromatin masses had been recently attached to corpuscles and were freed in the form which they assumed when attached. The parasites with the two chromatin masses were two parasites, which had been attached to one corpuscular mound.

In studying migrations it will save time to work with the heavy infections.

*Free Parasites.*

Free parasites may be found in the blood of all malarial infections. In the æstivo-autumnal infections one may find free in the blood, round bodies and crescents, and parasites in the early stages of development. Instances of multiple infection of red corpuscles are more frequently seen in æstivo-autumnal infections than in other malarial infections. Where more than one parasite is attached to a corpuscle, they are set free prematurely by destroying the infected corpuscle sooner than one parasite would.

Free parasites may be (*a*) compact, (*b*) ring-form, or (*c*) ameboid, and they may show delicate filaments arising from the cytoplasm of the parasites for the purpose of attachment to fresh red corpuscles. Sometimes these filaments are so delicate as to be scarcely discernible and one's attention is first attracted to a pigment granule in connection with a filament so fine that otherwise it would not have been noticed.

Almost every worker who has done much research in connection with malarial parasites has seen parasites free, either in fresh or stained specimens. They have been so frequently seen and described that it seems surprising that a true interpretation of their presence in the blood did not suggest itself.

[16] Bignami, A., and Bastianelli, D. G., quoted from Thayer, W. S., Lectures on the malarial fevers, New York, 1897, 168.

[17] Macfie, J. W. S., *Lancet*, 1914, ii, 1354.

Laveran[18] writes of the parasites as "now free in the blood, now adherent to blood corpuscles." Mannaberg[19] has observed free not only "spores," but "parasites which are developed further than their spore-forming stage," and he states that the way their structure takes the stain is so striking that one is prevented from suspecting them to be degeneration forms, or from mistaking them for other blood elements. Canalis[20] has seen free parasites in æstivo-autumnal infections. Thayer[21] describes parasites as free in the cerebral capillaries. Marchiafava and Bignami[22] have observed free parasites. DaCosta[23] describes young parasites as escaping prematurely from red corpuscles. Ewing[24] states that, associated with intracellular parasites, he frequently saw young æstivo-autumnal parasites free in the plasma. Celli and Guarnieri[25] sketched the appearance of young extracellular bodies, including forms of at least two species of parasite. Gautier and Ziemann[26] have observed free parasites. Councilman and Abbott[27] described pigment-bearing bodies in and outside red corpuscles. Golgi[28] states that sometimes parasites may be seen free in the plasma. Ewing[29] must have attached a certain importance to the fact that free parasites were seen in the blood, for he has written of them under the heading of "Extracellular parasites."

The parasites illustrated in this article were secured from two cases of æstivo-autumnal infection.

[18] Laveran, A., Plaudism, translation by Martin, J. W., London, 1893, 12.

[19] Mannaberg, J., The malarial parasites. A description based upon observations made by the author and by other observers, translation by Felkin, R. W., London, 1894, 277.

[20] Canalis, P., quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 22.

[21] Thayer, W. S., Lectures on the malarial fevers, New York, 1897, 61.

[22] Marchiafava, E., and Bignami, A., in Stedman, T. L., Twentieth century practice, New York, 1900, xix, 291.

[23] DaCosta, J. C., Clinical hæmatology, Philadelphia, 1901, 365.

[24] Ewing, J., *J. Exp. Med.*, 1900–01, v, 446.

[25] Celli and Guarnieri, quoted from Ewing, J., *J. Exp. Med.*, 1900–01, v, 473.

[26] Gautier and Ziemann, quoted from Ewing, J., *J. Exp. Med.*, 1900–01, v, 473.

[27] Councilman and Abbott, quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 12.

[28] Golgi, quoted from Thayer, W. S., and Hewetson, J., *Johns Hopkins Hosp. Rep.*, 1895, v, 16.

[29] Ewing, J., *J. Exp. Med.*, 1900–01, v, 472.

SUMMARY.

The anemia in malarial infections is explained by the fact that each parasite destroys several red corpuscles.

Reduction of hemoglobin out of proportion to the loss of red corpuscles is explained by the fact that there is always a partial loss of hemoglobin in certain of the surviving corpuscles due to parasitic action.

Migration of parasites occurs in all æstivo-autumnal infections. If one wishes to observe migrations, it will save time to study the heavy infections.

Free parasites have been frequently noted by many observers, who have failed to interpret properly their presence in the blood.

## EXPLANATION OF PLATES.

### PLATE 17.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,780.

FIGS. 1 to 30. Free parasites in migration. The pigment granules (at o) are evidence of previous attachments.

FIGS. 31 to 34. Young parasites on corpuscular skeletons. In Fig. 34 a pigment granule is shown at o.

### PLATE 18.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,780.

FIGS. 35 to 37. Free parasites with attaching pseudopodia arising from the cytoplasm of the parasites. Pigment granules (at o) are evidence of previous attachments.

FIG. 38. Three young parasites freed from one corpuscle.

FIGS. 39 to 41. Young parasites with attaching pseudopodia. Pigment granules indicating previous attachments may be seen at o. In Fig. 41 the filament connecting the pigment granule with the parasite is scarcely discernible.

FIGS. 42 and 43. Instances of two young parasites freed from one corpuscle. In Fig. 42 a pigment granule is seen at o.

FIGS. 44 to 58. Free ameboid young parasites. Pigment granules at o. Fig. 57 shows two free parasites; chromatin masses at x.

FIG. 59. Two free parasites; one is ameboid, the other at x is compact.

FIGS. 60 to 63. Instances of two young parasites freed from one red corpuscle. Note that at o pigment granules may be seen in connection with the parasites.

Figs. 60, 62, and 63 show pigment granules in connection with but one of the parasites. In Fig. 63 the parasite at x is compact.

Fig. 64. Three young parasites probably freed from one red corpuscle. The two at x are compact.

## Plate 19.

### ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,780.

Fig. 65. A young parasite attached to a red corpuscle almost entirely dehemoglobinized, and an older parasite attached to a peripheral mound of a healthy appearing corpuscle. At x a young parasite is seen attached to a decolorized corpuscle.

Figs. 66 to 69. These figures show young parasites free and also instances of two parasites attached to one corpuscular mound (at x). Fig. 67 shows two pigment granules at o.

Figs. 70 to 79. Young parasites attached to fairly healthy appearing red corpuscles, the bodies of the parasites resting on the periphery of the corpuscle. Fig. 74 shows a pseudopodium arising from the cytoplasm of the parasite.

Figs. 80 to 84. Young parasites attached to peripheral corpuscular mounds of healthy appearing red corpuscles.

Fig. 85. A young parasite attached to a corpuscle by a delicate pseudopodium.

Figs. 86 to 91. Parasites partly off decolorized red corpuscles. Pigment granules may be seen at o. In Fig. 89 a compact parasite is seen at x. In Fig. 91 two young parasites are seen attached to a healthy appearing corpuscle, while one parasite has decolorized the corpuscle at their right.

Fig. 92. A young parasite attached to a peripheral mound of a decolorized red corpuscle. Part of the parasite extends beyond the periphery of the corpuscle. A pigment granule is seen at o.

Fig. 93. A young parasite attached to a decolorized corpuscle.

# THE FUNCTIONAL VALUE OF NEWLY FORMED CONNECTIVE TISSUE.

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Experimental tuberculosis, viewed broadly, presents a characteristic picture not to be confused with any other except that of the spontaneous or natural disease. Experiments devised to analyze the picture into its component parts have usually failed to develop information of decisive value. If, for example, parallel inoculations are made with living tubercle bacilli and with those killed by heat, the lesions produced are much alike in the beginning and remain so for some time. Finally, those due to the living bacilli are progressively reduplicated by the further invasion of the tissues, while those due to the dead bacteria are gradually healed. It is difficult again to say in what essential particular the lesions due to dead tubercle bacilli differ from those caused by other mildly irritant foreign bodies introduced into the tissues.

The lesions above mentioned have in common an initial acute inflammatory reaction and a subsequent deposit of more or less perfectly formed fibrous connective tissue. At the point of divergence this connective tissue in the case of the living tubercle bacillus undergoes degenerative changes; it is broken down. But this divergence in the type of reaction is not universal. With the living bacteria, wherever either locally or generally the infection is resisted or overcome the connective tissue develops more or less steadily until the products of the earlier inflammation are replaced. It then tends to be reabsorbed. If the mass is small it may completely disappear with time; if the mass is large it remains as a scar in no essential particular to be distinguished from a scar due to any other cause.

This reaction of the connective tissue, intimately associated as it is in favorable cases with the healing of tuberculosis, may in the abstract be viewed either as a process dependent in its entirety on other

factors in the disease or it may be considered to be a factor of prime importance in the recovery or partial recovery of the animal. Facts are lacking on which to base the adoption of one or the other of these concepts. For the purposes of this paper, and merely as a working hypothesis, we have adopted the view that the reactive qualities of the connective tissue may be the decisive factor in determining the outcome of a tuberculous infection.

In order to proceed from this point we require some knowledge not at present available of the connective tissue and its reactive possibilities. Is the connective tissue in this sense a constant or a variable for any species of animal? If connective tissue is to be held responsible for variations in reaction to the tubercle bacillus it can only be on the assumption that it has a variable capacity for reaction. Such an assumption is, of course, in accord with the presumption on the broadest biological principles, most, if not all, functions which can be measured being variants in either direction from a typical mean value. In the search for a measure of the capacity of connective tissue to react we have turned to the facts and experiments relating to the healing of wounds.

The repair of any solution of continuity of tissues other than the purely epithelial surfaces involves the production of connective tissue, and attempts have been made to study this reaction quantitatively, particularly, of recent years by Carrel and his associates.[1] A careful consideration of the conditions of these studies makes it seem unlikely that the values determined have any direct relation to the reaction of connective tissue.

As an example we may select an incised wound made through the skin into the soft parts and allowed to gape during the healing process. The gaping wound will fill with exudate which will then become organized with granulation tissue, and this will subsequently be replaced by connective tissue. When the gape or cavity has become well filled with granulations the epithelium will begin to cover the wound by extension from the margins. It is the rate of growth of the epithelium which has been the subject of the quantitative studies above mentioned. Granted that this growth of the epithelium is dependent

[1] Carrel, A., du Noüy, P. L., and Carrel, A., Cicatrization of wounds. IX, *J. Exp. Med.*, 1917, xxvi, 297.

on the state of the underlying connective tissue or granulation tissue as the case may be, it is yet most unlikely that the rate of epithelial growth indicates either the rate of the preceding repair of the subcutaneous tissues or its functional perfection. If conditions could be precisely defined it is probable that in the whole process of wound healing the period when the deep defect was being replaced by potential connective tissue would take its place as a latent period in the reaction of the epithelium.

We have found one account in the literature of an experiment made to measure the effectiveness of connective tissue repair. Sir James Paget[2] (1853) writes as follows:

"The strength, both of the new substance itself, and of its connection by intermingling with the original substance, is worthy of remark. To test it, I removed from a rabbit an Achilles tendon, which had been divided six days previously, and of which the retracted ends were connected by a bond of the size and texture usual at that period of the reparative process. I suspended from the half-section of this bond gradually increased weights. At length it bore a weight of ten pounds, but presently gave way with it: yet we may suppose the whole thickness of the bond would have borne twenty pounds. In another experiment, I tried the strength of a bond of connection which had been ten days forming: this, after bearing suspended weights of twenty, thirty, forty, and fifty pounds, was torn with fifty-six pounds. But surely the strength it showed was very wonderful, when we remember that it was no more than two lines in its chief diameter; and that it was wholly formed and organized in ten days, in the leg of a rabbit scarcely more than a pound in weight. With its tenacity it had acquired much of the inextensibility of the natural tendon. It was indeed stretched by the heavy weights suspended from it, yet so slightly that I think no exertion of which the rabbit was capable would have sufficed to extend it in any appreciable degree."

While these experiments were directed toward a study of the repair of tendons a superficial review of the conditions suffices to show that in the early days following tendon section it must be a value for connective tissue which is determined. This connective tissue may or may not be wholly representative, but it is difficult to devise a better experimental test for the reactive capacity of connective tissue. We therefore adopted the general method of Sir James Paget's experiments as a basis for further work. Our work falls naturally into

[2] Paget, J., Lectures on surgical pathology, London, 1853, i, 271.

two phases: (1) an effort to determine the rate of replacement of the tendon as measured by the strength of the connective tissue laid down between the cut ends; (2) an attempt to use the method in the study of the action of certain chemicals locally or generally applied and for the examination of that phase of the tuberculosis problem previously discussed.

*Method.*

Adult rabbits were anesthetized with ether. With various degrees of aseptic precaution the Achilles tendon was exposed and sectioned. At various intervals thereafter the animal was killed and the leg amputated at the hip and suspended by the knee through a strong wire loop as an intermediary to a stout chain hung from the ceiling. With a similar wire loop a pail was suspended from the hock. Into this pail clean sand was run from a funnel at a uniform rate and from a uniform height. When a break occurred the flow of sand was stopped, the pail, its contained sand, and all the apparatus coming away with the foot were weighed, the result being recorded in grams as the tensile strength of the newly formed tissue.

At the time the leg was amputated the tendon was dissected free from the surrounding tissue and covered with gauze wet with salt solution. After the leg was hung up, as a last step before the sand was run in, the tibia and the remaining soft parts were cut through with bone forceps and scissors. In this way the new tissue was protected against any irregular strain during the time of preparation.

In general the method can be said to have served its purpose. The difficulties and irregularities for the most part seem to be connected with the surgical procedure. Infection must be avoided as the presence of small pockets of pus in the connective tissue naturally weakens it. Hemorrhage at the time the tendon is cut cannot always be controlled and when the test is made the instances in which evidence of hemorrhage persists must be excluded. The tendon sheath must be carefully sutured at the time of operation. If this is not done the exudate extends through the opening and infiltrates the surrounding fascia. This then organizes and forms adhesions which may involve all the structures from the newly formed matrix of the tendon outward to the skin. On dissection it then is impossible to

isolate the representative tissue. The new connective tissue seems to spring from the old tendon sheath as well as from the cut ends of the tendon, or at least adhesions to the old tendon sheath are universal. If when the dissection is done an attempt is made to remove the last traces of old connective tissue, splits and shreds will be run down into the new tissue which would naturally weaken it. With care, however, the old tissue can be reduced to a thin film without damage to the new. It is not difficult to avoid the gross sources of error, and the variations due to minor faults probably do not affect the results in the main.

*Experiments Bearing on the Healing of Wounds.*

In the first sets of experiments, those intended to determine the general feasibility of the method, surgical cleanliness was sought through the use of antiseptic solutions. A coal-tar disinfectant of the creolin type was used in solution of appropriate strength for cleansing the skin, keeping the hair wet, and cleansing the hands and instruments. Ordinarily care was taken to touch the field of operation with instruments only and these were freed from excess of antiseptic. Protocols selected as being representative are summarized in Tables I and II.

The experiments recorded in Tables I and II were, as stated above, carried out depending upon an antiseptic solution for surgical cleanliness. In Table III is shown the result of an experiment in which the operative work was done with rigid aseptic precautions. All material used was previously sterilized in the autoclave. Gloves were worn and at the same time especial precautions were taken to keep dust and bits of hair from the skin of the animals from contaminating the wound. In this experiment dichloramine-T is again introduced.

It is not our present purpose to discuss the results of the three experiments presented so far with any pretense to finality.

The results of the second series are in accord with the expectation we entertained when benzene was administered to part of the rabbits. Benzene is known to cause an extensive aplasia of the lymphoid tissue and to give rise to an extreme leucopenia. It was expected to influ-

ence in a deleterious way the exudation processes involved in the repair or replacement of the tendon. The experiment, if its results are accepted at their face value, and we see no reason why they should not be so accepted, indicates that the method is adequate in that it is capable of revealing the action of extraneous influences brought to bear on the reparative process through systemic channels.

It will be noted that there is a large difference between the average tensile strength found in Tables II and III and that that in

TABLE I.

The Achilles tendons of rabbits were cut by open operation under ether anesthesia. The tendon sheath and skin were sutured separately. In the dichloramine-T series 7½ per cent dichloramine-T dissolved in chlorinated eucalyptol, specific gravity 1.2, was put into the tendon sheath and the remainder of the wound at the operation. A small amount remained when the sutures were tied. Breaking strength determined 10 days after section of tendons.

| Untreated. | Dichloramine-T. |
|---|---|
| *gm.* | *gm.* |
| 4,120 | 5,470 |
| 6,320 | 7,170* |
| 6,620 | 7,520 |
| 7,750 | 9,100 |
| 7,900 | 9,370 |
| 8,050 | 10,520 |
| 10,070 | 12,200 |
| 10,600 | |
| Average..... 7,678 | 8,764 |

* In this case the connective tissue did not break. The tendon pulled out of the muscle and the recorded strength is considerably too low.

Table I is intermediate to these. The low place of Table II is in part to be explained by the fact that when the first of the series were tested the operator made more effort to trim away all traces of the original connective tissue than has seemed practicable. In order that the experiment might not be lost the method was persisted in even though it seemed somewhat faulty. It is probably true also that there was in this series in general more evidence of slight persistent hemorrhage and low grade infection than in the others. The internal varia-

TABLE II.

Operation as in Table I. The rabbits in the benzene series had 5 cc. of 50 per cent benzene in olive oil administered subcutaneously on the day after operation. Breaking strength determined 7 and 10 days after operation.*

| Untreated. | | Benzene. | |
|---|---|---|---|
| 7 days. | 10 days. | 7 days. | 10 days. |
| *gm.* | *gm.* | *gm.* | *gm.* |
| 2,270 | 3,900 | 1,900 | |
| 2,920 | 4,140 | 2,380 | |
| 3,120 | 4,400 | 2,430 | |
| 3,450 | 5,050 | 2,540 | 2,240 |
| 3,650 | 5,350 | 2,650 | 2,540 |
| 3,850 | 5,700 | 2,720 | 3,350 |
| 3,950 | 5,970 | 2,950 | 3,390 |
| 4,200 | 6,120 | 3,420 | 3,970 |
| 4,950 | 6,200 | 4,170 | 5,750 |
| 5,620 | 6,650 | 4,270 | 6,200 |
| Average. 3,798 | 5,348 | 2,943 | 3,917 |

* This whole experiment was carried out by Mr. R. H. Kennicott, then a special student in the Medical School of the University of Pennsylvania.

TABLE III.

Operation aseptic; otherwise as in Table I. In the dichloramine-T series the statements made in Table I about similar series also apply. Breaking strength determined at 10 days.

| Untreated. | Dichloramine-T. |
|---|---|
| *gm.* | *gm.* |
| 4,920 | 4,350 |
| 6,450 | 5,350 |
| 7,150 | 6,650 |
| 7,700 | 7,300 |
| 8,170 | 7,600 |
| 9,150 | 7,650 |
| 10,000 | 7,850 |
| 11,170 | 8,800 |
| 12,750 | 10,000 |
| 13,750 | 12,800 |
| Average..... 9,121 | 7,835 |

tion in Table II is, however, no greater than the others, if it is as great.

The increased strength attained in 10 days in Table III contrasted with Table I is, we believe, accounted for by the improvement in operative technique—a careful aseptic method having been substituted for a rather crude procedure in which reliance was placed in an antiseptic solution. The fourth experiment (to be presented later), also done aseptically and after more experience, shows a still further increase in the average tensile strength.

In contrasting these experiments emphasis has been laid on the averages. A scrutiny of the tables shows that the averages are surprisingly representative. Where the average is higher it is not due to the presence of exceptionally high figures for individuals.

The dichloramine-T results are not such as to permit satisfactory interpretation. They might be taken to indicate that this material helped in the case of an imperfectly carried out antisepsis and hindered healing when asepsis was better. This substance is, in any event, not one which would be recommended to be left in wounds which are closed tightly as it is capable of causing an aseptic inflammation in the solvent used. Our purpose in including these figures at present is to make available all our data.

As a general method for the quantitative study of a fundamental aspect of wound healing we cannot feel that we have contributed anything in finished form. The wide variation in values found necessitates work on a statistical basis and if there prove to be no other limitation the method is obviously laborious.

### *Experiment on Tuberculosis.*

Having in mind the possible place of the reactivity of the connective tissue in relation to the healing of tuberculosis as presented in the introductory paragraphs of this paper, we sought information by the application of the method as developed to the problem along the following lines. The fact that the length of life of animals of a given species inoculated with anything less than an overwhelming dose of virulent tubercle bacilli varies widely has recently been emphasized by the work of our laboratory. Whatever the reason may be the

strength of the newly formed connective tissue as determined above also varies over a wide range. We sought to determine whether the variations in the two cases were parallel or otherwise. With this purpose in mind the following experiment was carried out.

Young well grown rabbits, weighing about 1,500 gm., were chosen. The Achilles tendon of one leg was sectioned. On the 10th day the leg was amputated at the hip and the tensile strength of the reparative tissue was tested as described. The amputation was carefully conducted under ether anesthesia. Time additional to that needed

TABLE IV.

The rabbits weighed approximately 1,500 gm. The tendons were cut between October 1 and November 15, 1917, under ether anesthesia. Leg amputated and tensile strength of connective tissue determined on the 10th day. March 1, 1918. Inoculated all surviving animals with 0.02 mg. of tuberculosis culture, Bov. C., intravenously.

| Length of life after inoculation of tubercle bacilli. | Tensile strength. | Length of life after inoculation of tubercle bacilli. | Tensile strength. |
|---|---|---|---|
| *days* | *gm.* | *days* | *gm.* |
| 43 | 8,220 | 62 | 10,500 |
| 45 | 6,420 | 62 | 8,200 |
| 45 | 12,500 | 62 | 6,850 |
| 45 | 9,600 | 85 | 7,550 |
| 48 | 12,600 | 88 | 14,000 |
| 51 | 11,400 | 92 | 13,100 |
| 52 | 11,550 | 92 | 14,700 |
| 55 | 8,300 | 92 | 8,800 |
| 57 | 9,070 | 113 | 11,200 |
| 58 | 13,100 | 113 | 9,900 |
| Average strength of first half to die..... | 10,276 | Average strength of second half to die..... | 10,480 |

for healing of the operation wound was allowed for the perfect recovery of the animal from the effects of this precedure. The animals were then inoculated intravenously with a suspension of virulent tubercle bacilli (0.02 mg. of the growth on glycerol agar). Every effort was made to attain uniformity in this inoculation. The animals were then retained until they succumbed to the tuberculosis. In Table IV the length of life of the rabbits in days after the inoculation with tubercle bacilli is set over against the tensile strength of the

connective tissue in grams. Calculation shows that the average tensile strength of the connective tissue formed by the first ten rabbits to die is practically identical with that formed by the last ten animals.

It is apparent that no correlation appears, that consequently we have by this experiment demonstrated no relation between the reactivity of connective tissue in a normal reparative process and the general resistance of the animal to experimental infection with *Bacillus tuberculosis*. In fact the experiment seems sufficiently decisive to warrant the tentative conclusion that no relation exists between any dominant factor in the laying down of connective tissue in response to traumatism and natural resistance to tuberculosis.

## SUMMARY.

1. A method has been revived which enables the connective tissue factor in wound healing to be studied quantitatively.

2. It has been found that the functional value of connective tissue formed in response to traumatism as represented by its tensile strength varies widely in different animals.

3. It is suggested that the method may find application in the study of extraneous influences which may affect wound healing either through local or systemic application.

4. It has been determined that there is no parallelism between an active connective tissue response to traumatism and natural resistance to inoculation tuberculosis in the rabbit.

# PERSISTENCE OF THE VIRUS OF POLIOMYELITIS IN THE NASOPHARYNX.

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This paper is intended as a contribution to the epidemiology of poliomyelitis. Present knowledge places that disease among the infections in which the specific cause is carried in the nasopharynx, and present belief is to the effect that the virus is conveyed from one person to another through the medium of the nasopharyngeal secretions. Indeed, the virus has been detected in these secretions by the inoculation test in three sets of conditions; (1) coincidentally with an attack of the disease incited by it; (2) a considerable period after the attack of the acute disease has abated; and (3) in healthy persons who have been in contact with cases of poliomyelitis.

This determination having been made, detailed information covering the frequency with which and the conditions under which the virus can be discovered in the nasopharynx is greatly to be desired. This information is essential to the working out of the principles of the method of control of epidemic poliomyelitis.

Account must be taken, at the outset, of the difficulties surrounding, at the present time, the demonstration of the virus. The only reliable means of its detection is the inoculation test. When an active virus is injected intracerebrally or even otherwise into monkeys a train of symptoms tends to be set up in these animals which closely resembles the symptoms present in man, the subject of poliomyelitis. Indeed, the analogy is even closer than this, because the histological changes arising in the central nervous organs of the monkeys are an exact counterpart of those present in fatal cases of the disease in human beings. We can now state unreservedly that when typical symptoms appear in the inoculated monkeys typical lesions will occur in their nervous organs.

There is no division of opinion among observers regarding the clinical and histological evidences of poliomyelitis in monkeys in instances in which the experimental disease conforms to frank cases of poliomyelitis occurring in man. There is, however, lack of agreement respecting a less typical experimental condition described by Kling, Pettersson, and Wernstedt.[1] The questions raised by these experimenters, who have introduced entirely new criteria into the subject, are of far reaching significance, because they affect the character of the evidence acceptable as indicating the presence of the virus of poliomyelitis in the nasopharynx. This aspect of the general subject will come under consideration in connection with the experiments to be reported and discussed in the present paper.

### *Review of the Literature.*

In order that the experiments to be described may take their proper place among the studies dealing with the mode of infection in poliomyelitis, a brief review of the literature, especially of that stressing the nasopharynx as the portal of entry and of exit of the virus, will be given.

That the virus, the existence of which at that time was merely suspected, is communicated by personal contact was the thesis which Wickman's[2] studies served to emphasize. But until Landsteiner and Popper[3] communicated the disease to monkeys no more precise definition of the mode of infection could be given. It will be recalled that Landsteiner and Popper originally conveyed the infection from man to monkey by the intraperitoneal route of inoculation and were thus able to reproduce poliomyelitis only in the first series of injected animals. The employment of the intracerebral route of inoculation by Flexner and Lewis[4] led to the discovery that the virus could be transmitted from monkey to monkey through an indefinite series, in course of which its activity or virulence for monkeys increased many fold. Moreover, still other portals of experimental infection were successively disclosed, such as the large nerves, subcutis, subarachnoid space, nasal mucosa, eye, and, although with far greater difficulty, the general blood.

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[1] Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État Stockholm*, 1912, iii, 4.

[2] Wickman, I., Beiträge zur Kenntnis der Heine-Medinschen Krankheit, Berlin, 1907.

[3] Landsteiner, K., and Popper, E., *Z. Immunitätsforsch., Orig.*, 1909, ii, 377.

[4] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 1639.

In addition, it was found by Leiner and von Wiesner[5] that the virus would penetrate the mucous membrane of the gastrointestinal tract of monkeys in which the motions of the digestive organs were for a time arrested by means of opium. It is noteworthy that aside from the intracerebral route the other modes of infection give more or less inconstant results, and that the external portal which is most favorable to the attack of the virus is the nasal mucous membranes.

The particularly favorable position afforded by the nasal route to infection is a fact the demonstration of which was indicated by circumstances surrounding the epidemiology of poliomyelitis in man. Flexner[6] pointed out that the not infrequent confusion of epidemics of poliomyelitis with those of cerebrospinal meningitis argued for a similarity not only in certain cardinal symptoms but also in the conditions under which the two diseases arise. Thus each disease attacked by preference infants and young children, although not wholly sparing older children and adults. Usually a single case appears in a family or home, but sometimes two cases, and less often three or more appear. These resemblances came to be further emphasized by the detection of the meningococcus carrier on the one hand and the demonstration of the ambulant and abortive cases of poliomyelitis on the other. Indeed, the single striking epidemiological point of difference relates to the seasonal prevalence which for poliomyelitis tends to be summer and autumn and for cerebrospinal meningitis winter and spring. Even this distinction is not absolute; we now know that winter epidemics of poliomyelitis occur, and circumstances favoring epidemics of cerebrospinal meningitis arise in the warm months of the year. The meningococcus carriers have been known for more than a decade; it is important to ascertain whether corresponding poliomyelitic virus carriers exist.

Experience having shown that a highly potent poliomyelitic virus may be secured by successive passages through monkeys, the way was opened for an experimental study of the nasopharynx as the portal of its entry into and exit from the body. The filterability of the virus, moreover, permitted its entire separation from the bacteria present in that locality. Flexner and Lewis,[7] therefore, crushed and extracted in salt solution the excised nasal mucous membrane removed from monkeys which succumbed to intracerebral inoculation of the virus and after filtration through a Berkefeld filter injected the fluid into other *rhesus* monkeys, thus inducing infection and paralysis. The corollary to this experiment, namely the infection of monkeys by direct inoculation of the virus into the nasal mucosa, was quickly supplied by Landsteiner and Levaditi.[8] The last test has been carried out successfully in several ways: by applying the virus to the abraded mucous membrane, by introducing it upon cotton tampons, and

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[5] Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, xxiii, 91.

[6] Flexner, S., *J. Am. Med. Assn.*, 1910, lv, 1105.

[7] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 535.

[8] Landsteiner, K., and Levaditi, C., *Ann. Inst. Pasteur*, 1910, xxiv, 833.

finally by Leiner and von Wiesner[9] by merely pencilling the mucosa without causing any demonstrable lesion whatever. It should be stated, also, that one or the other of the modes of inoculation succeeds or not according, apparently, to the initial potency of the virus and the species of monkey chosen. Finally, some experimental evidence has been adduced to show that the nasal membranes are better suited to convey the virus than the tonsillar tissue. Levaditi and Danulesco[10] made submucous injections into the two regions and induced infection in the one case and not in the other.

The virus of poliomyelitis exists not only in the tissues but also in the secretions of the nasopharynx of monkeys, as shown by Landsteiner, Levaditi, and Danulesco,[11] who inserted cotton plugs into the nares of paralyzed animals and found that after several hours the absorbed fluid sufficed, when injected, to infect other monkeys; while Thomsen[12] ascertained also that merely rubbing the mucosa with the tampon was sufficient to incite infection. In this connection mention may be made of the fact that Landsteiner and Levaditi[8] consider that they have traced the passage of the virus along the olfactory nerves to the olfactory lobe of the brain, and Flexner and Clark[13] likewise have found the olfactory lobes infective, and the spinal cord and medulla non-infective, 48 hours after an intranasal inoculation of the virus. The last observation indicates that the penetration of the virus from the surface to the interior of the nasal membrane and thence to the brain occurs quickly, a point borne out by certain experiments made with antiseptic drugs to be reported elsewhere.

The foregoing experimental results provide a basis for considering the inoculations which have been made directly with human materials derived from the nasopharynx. These materials may be divided into three classes as follows: (1) washings, (2) tissues (tonsils and adenoids) removed during life, and (3) tissues (tonsils, pharyngeal mucosa, nasal mucosa) recovered after death. As regards the first, numerous tests have been made. It will be of doubtful value to note in this place the failure to induce infection. The negative results are, in the light of present knowledge, without real significance. The limits of activity for monkeys of virus immediately obtained from human beings are quickly reached. Hence the dilute filtered washings of the nasopharynx could be expected to succeed only rarely. We have ourselves failed to incite infection with a filtrate prepared from the spinal cord of human cases of poliomyelitis when the unfiltered suspensions were active.

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[9] Leiner, C., and von Wiesner, R., *Wien. klin. Woch.*, 1910, xxiii, 323.

[10] Levaditi, C., and Danulesco, V., *Compt. rend. Soc. biol.*, 1912, lxxii, 606.

[11] Landsteiner, Levaditi, and Danulesco, *Compt. rend. Soc. biol.*, 1911, lxxi, 558.

[12] Thomsen, O., *Berl. klin. Woch.*, 1912, xlix, 63.

[13] Flexner, S., and Clark, P. F., *Proc. Soc. Exp. Biol. and Med.*, 1912–13, x, 1.

However, Kling, Pettersson, and Wernstedt[1] have brought indubitable proof that washings obtained from the nasopharynx both during life from typical cases of poliomyelitis and after death from persons who succumbed to the disease, may carry the virus in a form and quantity capable of inciting infection in monkeys. If we confine ourselves strictly to the instances in which the washings conveyed typical experimental poliomyelitis, it may be said that while the number of failures to infect exceeds the successful inoculations, yet the latter comprise a convincing series. While opinion on this point can hardly be divided, the same cannot be stated for the interpretation which they place upon the results of the inoculation of washings from abortive cases, mere contacts, and from recovered persons, to which fuller reference will be made presently.

The first to detect the virus in tonsillar and pharyngeal mucosa obtained from a fatal case of poliomyelitis in man and thus to confirm Flexner and Lewis' experiments on the monkey were Landsteiner, Levaditi, and Pastia.[14] Flexner and Clark[15] about the same time reported several similar successful inoculations. The latter authors drew attention to the fact that when they injected filtrates of the tonsillar and nasal tissues infection failed; but when the same materials were rendered bacteria-free with 0.5 per cent phenol and inoculated as suspensions infection followed.

Thus far all the successful inoculations of the virus of poliomyelitis noted upon monkeys have been secured with materials obtained from recent typical cases of the disease in man or the experimental reproduction of that disease in those animals. An experimental answer to the important question of the period of the survival of the virus in the nasal and buccal mucosa was first successfully attempted by Lucas and Osgood,[16] who were still able to determine its presence in the experimentally infected monkey at the expiration of nearly 6 months.

Wickman's important studies[2] pointed unmistakably to the part played by so called abortive cases of poliomyelitis and healthy carriers of the microbic cause in disseminating poliomyelitis. The discovery of the experimental disease in monkeys led to the search for the virus in the nasopharynx of those two classes of persons. By far the most extensive inoculation tests published are those of Kling, Pettersson, and Wernstedt.[1] They even go beyond the phases of the subject indicated and include an investigation of the period of survival of the virus in the nasopharynx. Unfortunately this highly important study is marred by the fact that the criteria of the experimental disease in monkeys which the authors came to adopt do not conform to those which all experimenters accept as distinctive of poliomyelitis in the monkey. The point is an essential one. A close review of their protocols has led us to doubt the validity of their conclusions.

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[14] Landsteiner, K., Levaditi, C., and Pastia, C., *Semaine méd.*, 1911, xxxi, 296.

[15] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvii, 1685.

[16] Lucas, W. P., and Osgood, R. B., *J. Am. Med. Assn.*, 1913, lx, 1611.

The inoculation experiments which form the basis of this paper deal with this aspect of the subject.

Meanwhile, it has been shown through inoculation in one instance by Flexner, Clark, and Fraser[17] that healthy persons may harbor the virus in their nasal and buccal secretions. The instance in which the demonstration was made related to the parents of a young child acutely ill with poliomyelitis. The contact, therefore, between the carriers and the case was an intimate one. This unquestioned observation was followed by a similarly conclusive one by Kling and Pettersson,[18] who, it may be remarked in passing, attribute failure to incite typical clinical and anatomical effects in their earlier experiments to the injections of insufficient amounts of the virus. Finally, Taylor and Amoss[19] have recorded two instances—one an abortive or non-paralytic case of poliomyelitis and the other a healthy child who subsequently developed frank paralysis—in which the concentrated nasopharyngeal washings inoculated into monkeys induced the typical experimental disease.

### *Nature of Materials and Interpretation of Experiments.*

The experiments which we are reporting were made with human materials of two sorts; namely, tonsils and adenoids removed during life and tonsils and pharyngeal mucosa excised after death. The object in all the experiments was to ascertain the presence or absence of the virus as far as this could be determined by the inoculation test. In this way it was hoped to throw light on the persistence of the virus in the nasal and buccal membranes and their discharges.

The outcome of the tests is not so consistent as we would wish. But the inconsistency which will appear is instructive, for not only does it point the limitations of the inoculation test, but it helps clear up, at the same time, the more indefinite clinical effects which sometimes follow the injections of supposedly virus-containing materials. It may be mentioned here that we did not confirm the experiments of Kling, Pettersson, and Wernstedt respecting an atypical variety of poliomyelitis in the monkey which they finally came to attribute to the inoculation of too little virus-containing washings. Our tests, as will appear, were carried out with tissues and with quantities that should have sufficed for infection had the virus been present in amounts to be expected and in an active state.

[17] Flexner, S., Clark, P. F., and Fraser, F. R., *J. Am. Med. Assn.*, 1913, lx, 201.
[18] Kling, C., and Pettersson, A., *Deutsch. med. Woch.*, 1914, xl, 320.
[19] Taylor, E., and Amoss, H. L., *J. Exp. Med.*, 1917, xxvi, 745.

The last considerations are pertinent because of the striking contrast in the results obtained with human tissues removed either by operation or post mortem. The obvious deduction is to the effect that the upper respiratory mucosa carries far more active virus in the latter instance. All the postmortem tissues inoculated by us were derived from acute cases. Analogy with other acute infections leads to the assumption that in the fatal instances there is more active multiplication of the virus than in cases which go on to recovery. This is perhaps the simplest interpretation to put upon our tests.

The discrepancy seems to illuminate the question as to the time of maximum infectivity in epidemic poliomyelitis. As far as observations made during the great epidemic, which prevailed in New York State and elsewhere in 1916, can be construed, the implication is to the effect that this period is relatively brief and is greatest early in the disease. It cannot be said, however, that this point is definitely established. As a matter of fact, the literature contains records which directly controvert this idea. The matter is perhaps one not to be settled absolutely but rather to be placed on a relative basis. What we need first to know are the conditions under which the virus of poliomyelitis can be definitely determined to be present in the nasopharynx. This question we have endeavored to answer by a study of tissues removed post mortem and of hypertrophied tonsils and adenoids extirpated during life from persons who had suffered a typical attack of poliomyelitis some weeks or months earlier. In comparing the two classes of tissue, attention must be given the fact that the former, that is the materials removed post mortem, come from cases earlier in the course of the disease.

The results of the inoculation of the tonsillar and adenoid tissues removed during life have also an important bearing on the question of the chronic carriage of the virus upon the nasopharynx. The test of Lucas and Osgood[16] showed that the virus may be demonstrable on the nasopharyngeal mucous membrane of the monkey as long as 5 months after infection had been experimentally induced. Flexner and Clark[20] demonstrated it in the nasopharyngeal mucosa of a monkey surviving the paralysis more than 4 weeks and failed, in the

[20] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, lvi, 585.

same animal, to detect it in the spinal cord. The large series of tests with washings from human cases made by Kling, Pettersson, and Wernstedt[1] on the basis of which they believe they have shown chronic carriage of the virus to be common, have never been subjected to a searching control with human tissues. Our experiments with surgically removed tonsils and adenoids may be regarded as covering this point. It happens, indeed, that our results are quite opposed to those of the Swedish observers and teach rather that the chronic carriage is, at least, exceptional. On the whole the epidemiological data are in conformity with our experimental results.

The Swedish authors studied nine convalescents over a maximum period of 7 months on the basis of which they formulated the following deductions: "that the secretion from the mucous membranes of the mouth and intestine of persons who have recovered (from poliomyelitis) has had the power of infecting monkeys still several months, in one case 204 days (nearly 7 months), after the onset of the illness, giving rise to an experimental poliomyelitis with fatal issue . . . . It was only in one case that we did not succeed in demonstrating the presence of the virus after the comparatively short time of 30 days."[21]

In interpreting this statement and in considering the discrepancy with our studies, it is necessary to take into account the immediately succeeding paragraphs:

"During the time occupied by the investigations, the virus had changed its character, so that it no longer caused inflammations with cellular exudations. Instead of this the degeneration of the nerve cells, the changes of the glia cells and the neurophagocytosis caused by the enlarged glia cells have been the characteristic changes. They have thus been of the same type as those appearing in the monkeys injected with secretions from abortive cases, and virus carriers, changes which . . . . we consider ourselves justified in assuming to be due to a less virulent virus. *The experiment also shows, that the microbe rather quickly—already after 8–14 days—loses its power of causing inflammatory exudations in the inoculated animals. This fact is of very great importance from a practical point of view since it perhaps gives us the right to assume that the virus, possibly rather soon after the termination of the acute stage, gets weaker.*"[22] (Author's italics.)

We propose now to present our results in the form of tabulations with such discussion as seems called for. The postmortem tissues

[21] Kling, Pettersson, and Wernstedt,[1] p. 159.

[22] Kling, Pettersson, and Wernstedt,[1] pp. 159–160.

employed for inoculation were obtained very soon after the death of the patient, when they were either inoculated after a short interval, or placed in the preserving fluids, which consisted of 50 per cent sterile glycerol or 0.5 per cent phenol. The tissues removed surgically were put immediately into the preservative fluids. The inoculations were made at leisure. The glycerolated or phenolized specimens were suspended and injected partly intracerebrally and partly intraperitoneally. Previous experiments had shown that the weak phenol destroyed associated bacteria in the tissues without acting appreciably upon the virus of poliomyelitis.[15] In this respect the phenol was superior to the glycerol, which also was without observed injurious action on the virus, but which removed the bacteria much more slowly.

Histological studies were made with the nervous organs of all monkeys succumbing to or after the inoculations. All the animals which developed typically clinical poliomyelitis showed characteristic histological lesions in the spinal cord and medulla and intervertebral ganglia. In none of the animals in which the clinical symptoms were dubious did we find histological lesions either resembling those of typical poliomyelitis or corresponding with the degenerative and peculiar neurophagocytic ones which Kling, Pettersson, and Wernstedt[1] describe and attribute to the action of specifically weakened poliomyelitic virus.

Examination of Table I brings out the fact that both the tonsils and nasal mucosa of fatal early cases of poliomyelitis are infectious for monkeys. No real distinction can be drawn between the two sets of materials as regards their infectivity. The fact that now one tissue from an individual succeeds while the other fails is probably as ascribable to differences in the susceptibility of the individual monkeys as to irregularity in distribution of the virus. The inferiority of filtrates to emulsions or suspensions of the tissues is manifest. It is clear also that phenol is a favorable medium for preparation of the tissues for inoculation, as it tends quickly to destroy bacteria associated with the virus without materially injuring the virus itself. Probably the 0.5 per cent phenol is only relatively innocuous for the virus, since in the instance of H. K., in which two *rhesus* monkeys were inoculated,

TABLE I.

*Human Tissues Obtained post Mortem.*

| Case. | Day of disease. | Monkeys inoculated. | Material injected. | Symptoms. | Histology. | Remarks. |
|---|---|---|---|---|---|---|
| J. A. R. | 6 | 2 *M. rhesus.* | Berkefeld filtrate of tonsils and pharynx mucosa. | None. | | Filtrate of spinal cord negative; emulsion positive. |
| R. P. | 7 | 2 *M.* " | (1) Emulsion of tonsils. | Tonsils none. | Typical lesions from nasal mucosa. | Glycerol preservation. Nasal mucosa positive. Tonsils negative. |
| | | | (2) Emulsion of nasal mucosa. | Nasal mucosa typical. | | |
| M. K. | 6 | 2 *M.* " | Heim filtrate of tonsils and nasal mucosa separately. | None. | | Glycerol preservation. |
| J. C. | 3 | 1 *M.* "<br>1 *M. cynomolgus.* | Heim filtrate of tonsils and nasal mucosa separately. | Nasal mucosa none. Tonsils typical. | Typical lesions from tonsils. | Glycerol preservation. Tonsils positive in *M. cynomolgus.* Nasal mucosa negative. |
| B. T. | 6 | 1 *M. rhesus.* | Emulsion of tonsils. | Typical. | Typical lesions. | Phenol preservation. Reinoculation of spinal cord positive. |
| J. G. | 7 | 1 *M.* " | " " " | " | " " | Phenol preservation. |
| S. A. | 9 | 2 *M.* " | " " " and pharynx mucosa separately. | None. | | " " |

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|---|---|---|---|---|---|---|
| H. K. | 6 | 2 *M. rhesus* | Emulsion of tonsils. | Typical, with recovery. | | Phenol preservation in successive quantities to remove resistant bacteria. |
| G. G. | 6 | 3 *M.* " | " " " | Typical. | Typical lesions. | Phenol preservation. |
| A. K. | 8 | 1 *M.* " | " " " | None. | | |
| J. W. | 4 | 1 *M.* " | " " " and pharynx mucosa. | " | | |
| G. C. | ? | 1 *M.* " | Heim filtrate of tonsils. | Indefinite. | Tuberculosis. | |
| B. H. | 10 | 1 *M.* " | Heim filtrate of tonsils. | None. | | |
| A. S. | 3 | 1 *M.* " | Emulsion of tonsils intrasciatic and intraperitoneal. | " | | Glycerol preservation. |

TABLE II.

*Human Tissues Removed Surgically.*

| Case. | Day of disease. | Monkeys inoculated. | Material injected. | Symptoms. | Histology. | Remarks. |
|---|---|---|---|---|---|---|
| H. G. | 8 | 1 *M. rhesus.* | Berkefeld filtrate of tonsils and adenoids. | None. | | |
| I. P. | 13 | 1 *M.* " | Emulsion of tonsils and adenoids. | Gradual emaciation with diarrhea. Died 15th day. | No lesions in nervous organs. | Phenol preservation. |
| A. S. | 13 | 1 *M.* " | Heim filtrate of tonsils and adenoids. | Indefinite. Recovered. | | |
| J. P. | 13 | 1 *M.* " | Emulsion of tonsils and adenoids. | None. | | Glycerol and phenol preservation. |
| H. B. | 14 | 1 *M.* " | Emulsion of tonsils and adenoids. | Emaciation. Died 15th day. | Tuberculosis. | Phenol preservation. |
| C. M. | 17 | 1 *M.* " | Filtrate of tonsils and adenoids. | None. | | " " |
| H. N. | 18 | 2 *M.* " | Emulsion of tonsils and adenoids. | " | | |
| C. U. | 19 | 2 *M.* " | Berkefeld filtrate of tonsils and adenoids. | " | | |
| J. A. | 19 | 2 *M.* " | Emulsion of tonsils and adenoids | Indefinite. | Tubercles in spinal cord. | Glycerol preservation. Succumbed to tuberculosis. |
| F. S. | 22 | 1 *M.* " | Berkefeld filtrate of tonsils and adenoids. | None. | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| J. W. | 25 | 2 *M. rhesus.* | Emulsion of tonsils and adenoids. | None. | | Glycerol preservation. |
| H. F. | 25 | 2 *M.* " | Emulsion of tonsils and adenoids. | (1) None.<br>(2) Pressure. Symptoms from which recovered. Then indefinite. Death on 12th day. | No lesions suggestive of poliomyelitis. No degeneration of nerve cells in medulla, cord, or intervertebral ganglia, and no cellular infiltration. | Glycerol preservation. Small sterile cyst at point of inoculation. |
| F. S. | 27 | 2 *M.* " | Emulsion of tonsils and adenoids. | None. | | Glycerol preservation. |
| L. F. | 27 | 2 *M.* " | Emulsion of tonsils and adenoids. | " | | Glycerol preservation. |
| A. L. | 27 | 1 *M. cynomolgus.* | Emulsion of tonsils and adenoids. | " | | Phenol preservation. |
| C. J. | 28 | 1 *M. rhesus.* | Filtrate of tonsils and adenoids. | " | | |
| H. F. S. | 28 | 2 *M.* " | Berkefeld filtrate of tonsils and adenoids. | " | | |
| A. S. | 35 | 1 *M.* " | Berkefeld filtrate of tonsils. | " | | |
| G. C. | 35 | 1 *M.* " | Emulsion of tonsils and adenoids. | " | | Phenol preservation. |
| T. P. | 39 | 1 *M.* " | Filtrate of tonsils and adenoids. | Indefinite. Recovered. | | " " |

TABLE II—*Concluded.*

| Case. | Day of disease. | Monkeys inoculated. | Material injected. | Symptoms. | Histology. | Remarks. |
|---|---|---|---|---|---|---|
| J. O'B. | 49 | 2 *M. rhesus.* | Emulsion of tonsils and adenoids. | (1) Died suddenly 8th day.<br>(2) Gradually lost strength. Died 15th day. | (1) No lesions in nervous organs.<br>(2) No lesions in nervous organs. | Glycerol preservation. |
| D. M. | 50 | 2 *M.* " | Emulsion of tonsils and adenoids. | (1) None.<br>(2) Indefinite. | (2) No lesions in nervous organs. | Glycerol preservation 5 days. Cyst in cerebrum at point of inoculation. |
| F. McK. | 50 | 2 *M.* " | Emulsion of tonsils and adenoids. | (1) Typical. Died 41st day.<br>(2) None. | (1) No lesions in nervous organs. | Phenol preservation. |
| T. M. | 90 | 1 *M.* " | Emulsion of tonsils and adenoids. | None. | | Phenol preservation. Reinoculation with emulsion. |
| J. W. | 90 | 1 *M.* " | Emulsion of tonsils and adenoids. | Died suddenly 2½ mos. after 1st and 19 days after 2nd inoculation. | No lesions in central nervous organs. | Phenol preservation. Reinoculation with emulsion. |
| M. K. | 120 | 1 *M.* " | Emulsion of tonsils and adenoids. | Indefinite after 2nd inoculation. Recovered. | | Phenol preservation. Reinoculation with emulsion. |
| E. S. | 150 | 1 *M.* " | Emulsion of tonsils and adenoids. | Indefinite after 2nd inoculation. Recovered. | | Phenol preservation. Reinoculation with emulsion. |

and in which the tissues employed were put through three successive solutions of the phenol to render them approximately bacteria-free, a milder form of poliomyelitis developed. The number of tests made is insufficient to account for certain obvious discrepancies as, for example, the failure of tissues derived from cases dying on the 3rd and 4th day of the disease to infect. Doubtless the fact that only one monkey was used in each instance for inoculation had something to do with the failures. But at times the supply of monkeys was too small and precarious to permit of more being tested. Experience with inoculations of the spinal cord and medulla from human cases has shown us that the failure to incite infection in a single *rhesus* monkey does not indicate lack of power to infect still other individual animals of the same species.

The deduction from the tests summarized in the table is to the effect that the nasal and pharyngeal mucosæ of persons succumbing to poliomyelitis during the 1st week or 10 days of the disease probably regularly contain the poliomyelitic virus.

A glance at Table II shows at once a fundamental difference in the results of inoculating the specimens removed surgically and those obtained at autopsy. The extent of the distinction is not greatly lessened by the probability that the tests carried out with the surgical tissues err on the side of negativity. That certain of the surgical specimens contained virus in some amount we think most probable. But the essential fact remains that under the conditions of the experiments they regularly failed to incite infection and paralysis in the monkeys.

The two sets of tissues, those removed post mortem and those removed during life, differed especially in one respect; namely, that the former came from cases of poliomyelitis in the 1st week and the latter later in the course of the disease. We are inclined to attribute to this circumstance, with which may be associated the tendency for microorganisms to multiply more freely in the last hours of life, the great differences in effects observed.

In order to favor the induction of infection with the specimens removed relatively late after recovery from the acute symptoms of poliomyelitis, the reinoculation method of Flexner, Noguchi, and

Amoss[23] was also employed. By this method it is possible to convert a subminimal infective dose of the virus into an effective dose. But still no success was achieved with the surgical tissues.

## DISCUSSION.

The epidemiology of poliomyelitis has still to be worked out in detail, as many factors governing the spread of the disease remain to be discovered. That the virus or microbic cause is communicated by personal contact is now generally admitted. That the virus occurs in the nasopharynx, which constitutes the chief locus of ingress and egress to and from the body is also conceded. The fact that the virus has been, if rarely, detected in healthy persons who have been in intimate contact with early cases of poliomyelitis, and even in certain individuals who have recovered from the acute effects of the disease, has led to the generalization that like some other diseases of bacterial origin, and notably epidemic meningitis, healthy and chronic carriers of the virus are frequent. This view has received its main support from Kling, Pettersson, and Wernstedt, whose studies we have discussed. A critical analysis of the basis of their contention fails, however, to carry conviction, and the doubt which has arisen as to the true interpretation of their results is deepened, we think, by our more searching tests.

The results of the experiments reported in this paper conform closely with clinical experience in the United States, at least, and especially with the observations made by epidemiologists in the course of the wide epidemic in New York State and elsewhere during the summer and autumn of 1916. The conclusion reached at that time was to the effect that the communicability of the disease was a phenomenon chiefly of the early stages, while the frankly paralyzed person and the convalescent were to be feared much less. In our experiments infection was secured with tissues obtained during the 1st week, approximately, of the disease but not at the later periods.

## CONCLUSIONS.

The virus of poliomyelitis occurs in the nasopharynx of man and monkeys.

[23] Flexner, S., Noguchi, H., and Amoss, H. L., *J. Exp. Med.*, 1915, xxi, 91.

In man it has been detected by the inoculation test in washings from acute cases, rarely in similar washings from healthy contacts, in the nasopharyngeal tissues obtained from fatal cases in the 1st week of infection, but rarely, if ever, from nasopharyngeal tissues removed surgically at later periods in the course of the disease.

In monkeys, also, the virus has been detected in the secretions from acute experimental infections, in the nasopharyngeal tissues derived from early cases, and rarely from cases several weeks or months after recovery from the acute symptoms.

The inoculation of tonsils and adenoids obtained from cases of undoubted poliomyelitis either yielded definite results in the form of typical paralysis and histological lesions in the central nervous organs of the monkeys injected, or no symptoms or lesions which could be confounded with poliomyelitis. The indefinite symptoms and atypical lesions described in a certain class of inoculated animals by Kling, Pettersson, and Wernstedt were not encountered in our experiments.

The deduction from the experiments reported is to the effect that the virus is regularly present in the nasopharynx in cases of poliomyelitis in the first days of illness, and especially in fatal cases; that it diminishes relatively quickly as the disease progresses, except in rare instances; and that it is unusual for a carrier state to be developed. Hence the period of greatest infectivity of patients would appear to be early in the disease, which is probably the time at which communication of the virus from person to person takes place.

Available evidence proves that healthy carriers of the virus occur. We do not, however, possess data which indicate the frequency with which carriage arises. The fact that even after a severe and wide epidemic, such as occurred in the United States in 1916, the disease may virtually disappear within 2 or 3 years, points to the probability that enduring carriers of the active virus, whether healthy or chronic, are of exceptional occurrence.

We are greatly indebted to Dr. Cornelius G. Coakley for many of the specimens of surgically removed tonsils and adenoids with which our experiments were performed. We desire to express sincere appreciation for his cordial cooperation.

# I. RENAL FUNCTION INFLUENCED BY INTESTINAL OBSTRUCTION.

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The literature on the intoxication of intestinal obstruction reveals the fact that no direct study has been made of the renal function in this condition. Yet, there is indirect evidence of functional impairment in spite of the fact that the present histological methods fail to show definite alterations in the kidney parenchyma.

Hartwell, Hoguet, and Beekman (1, 2) report finding degeneration of the tubular epithelium at autopsy in many of their dogs dying from experimental obstruction of the intestine, but we assume that these changes were post mortem or were due to some secondary factor or to antecedent disease, for no demonstrable kidney lesions have been found in animals autopsied immediately after death from uncomplicated obstruction in this laboratory.

Indirect evidence of functional derangement is found, however, in the rapid increase in the non-protein nitrogen of the blood during the intoxication, as first reported by Tileston and Comfort (3) in a small number of human cases of obstruction and observed experimentally in dogs with isolated loops of the small intestine or with simple obstruction, by Cooke, Rodenbaugh, and Whipple (4). These observers have shown that the non-coagulable nitrogen of the blood rises rapidly to a high level, often from 100 to 300 per cent above the normal, which level is sustained until death in fatal cases, or until the clinical symptoms of intoxication have subsided in those animals that recover. The creatinine nitrogen shares decidedly in this rise as shown by these same experiments (4), and in this connection we wish to refer to the conclusion of Myers and Lough (5) that: "The creatinin rises above 2.5 mg. per 100 cc. of blood almost without exception only in conditions with renal involvement." In their studies these observers were dealing with cases of true nephritis in which the creatinine of the blood had perhaps accumulated over a relatively long period of time because of a failure on the part of the kidneys to eliminate it at the normal rate. In the case of acute intestinal obstruction, on the other hand, a much more rapid rise in the various non-protein nitrogenous constituents of the blood is made possible by the greatly increased rate of tissue protein catabolism. Upon removing the source of the intoxication it is found that the blood non-protein nitrogen soon

returns to normal again. In spite of this difference, however, between the case of true nephritis and what has been observed in acute intestinal obstruction, the possibility remains that the high values found for the creatinine of the blood in the latter condition may be due in great part to its retention by temporarily disabled kidneys.

At the time that observations (4) were made showing the high blood non-protein nitrogen in intestinal obstruction, it was observed that the level of urinary nitrogen excretion was very high, at times twice or three times normal. This great increase in nitrogen elimination indicated that a large excess of body protein was being broken down and it was thought that this phenomenon might explain the high non-protein nitrogen of the blood. However, it was shown in later experiments (6) that this increased protein breakdown gave the usual products of the normal protein catabolism, observed, for example, after protein feeding. It could not be denied that there was a possibility that small amounts of toxic split products might be formed by this abnormal protein catabolism, but present methods of analysis give no positive evidence for this. In view of the fact that there was no positive proof that toxic substances were formed from the excess protein catabolism, it became very difficult to give any satisfactory explanation for the heaping up of non-protein nitrogen in the blood. It was recalled that the normal kidneys can excrete enormous amounts of normal nitrogenous end-products and even this great protein catabolism of obstruction should be easily taken care of by a pair of normally functioning kidneys.

The above brief statements, therefore, indicate a lack of definite information regarding the eliminative function of the kidneys during periods of intoxication due to intestinal obstruction. It was with the purpose of supplying this information that the present study was undertaken.

## *Methods.*

Dogs, mostly females, were used in all the experiments. Only healthy young adult animals were selected after being under careful observation for several days at least, during which time the urine was examined with reference to its specific gravity, reaction, and albumin and cast content.

The dogs chosen were then allowed in most instances to fast several days (3 to 5) after which various renal function tests were performed on them in exactly the same manner and under the same general conditions which were to obtain in the later experiments. The data recorded at this time for each dog afford a control for the

later experiments on the same animal. Following this preliminary examination of the animal, a simple obstruction of the small intestine was made by section of the intestine with inversion of the cut ends. In Dog 19–55 (Tables VIII and IX) an isolated loop of the ileum was made in the usual way and the remaining ends of the intestine were united by a lateral anastomosis.

Methods for the measurement of renal efficiency were chosen which are simple to perform but which give a reasonably accurate idea of the activity of the kidneys at the moment of examination. None of the longer tests involving special diets or the administration of various substances by mouth could be employed because of the tendency to vomiting and diarrhea during the intoxication.

Since there exists a general concensus of opinion among those who have made a comparative study of the various renal function tests (7, 8, 9) that no single test so far devised is entirely adequate to detect every type of kidney impairment and to distinguish renal injury from the extrarenal factors often involved, we have selected the three following methods: (1) that of measuring the urea-excreting capacity of the kidneys; (2) the phenolsulfonephthalein elimination method of Rowntree and Geraghty (10, 11); (3) that of determining the rate of excretion of injected sodium chloride.

The first method, that of measuring the power of the kidneys to excrete urea, was carried out by determining the blood urea and simultaneously the rate of urea excretion in most instances after the injection of urea. These values have been expressed in the form of the ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

after the method of Addis and Watanabe (12) and Watanabe, Oliver, and Addis (13). These workers propose the use of this simple ratio after the administration of urea for determining the quantity of functioning renal tissue. Under the strain imposed by the administered urea, the kidney with a diminished amount of secreting tissue reveals its lack of reserve power by a lowering of the ratio. They found that the ratio is not lowered by the removal of one kidney in a

normal animal unless urea is given, when it becomes apparent that the maximum capacity to excrete urea is exceeded more readily after the secreting tissue has been cut down one-half.

In the present study there is no process comparable with that of removing a part of the cells and leaving the intact cells in a perfectly normal condition as in the experiments of Addis and Watanabe. Nevertheless, if a large number of the cells are injured so that their individual ability to function is impaired, the effect upon the ratio should be the same, and, if this impairment is very marked, the ratio should fall even when no urea is injected. The urea of both the blood and the urine was determined in our experiments by the Van Slyke-Cullen modification of Marshal's urease method (14, 15).

The phenolsulfonephthalein test was applied according to the procedure outlined by its authors, Rowntree and Geraghty (11). Often during the period of intoxication isotonic saline or glucose solution was injected intravenously and in every case the animal received more fluid during this time than during the corresponding control period, in order that the volume of fluid lost to the body by way of the alimentary tract might be made up in part at least.

In the third method, that of measuring the efficiency of the kidneys in excreting sodium chloride, the salt was injected intravenously in hypertonic solution and its excretion over short periods was followed, as in the case of the injected urea, both before and during the period of intoxication. The total chlorides of the urine were determined by the McLean-Van Slyke method (16).

During the periods of observation the dog was placed in a large metabolism cage fitted with a simple harness which holds the animal during catheterization in a comfortable, fixed position, thereby making this process easy to perform by one person in a few minutes time. The bladder was thoroughly rinsed with lukewarm distilled water just before and at the end of each period. Collection of samples was always begun a few minutes before the end of the period in order that it could be discontinued sharply at the expiration of the time allotted. In cases of diuresis following the injection of urea or saline solution the bladder was catheterized more frequently so that the samples could be collected directly. Night samples alone in the case of the chloride tests were collected in metabolism cages.

## EXPERIMENTAL OBSERVATIONS.

Not all the experimental data are submitted because some of the experiments were incomplete or complicated by factors not intended to be present which introduced difficulties in the interpretation of results. We may say, however, that the other experiments which are not tabulated confirm the points made in this communication.

### TABLE I.

*Dog 18–138. Simple Obstruction of the Ileum. Blood Urea. Phenolsulfonephthalein Elimination.*

| Date. | Weight. | Blood urea per 100 cc. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|
| *1918* | *lbs.* | *mg.* | *per cent* | |
| June 18 | 40.5 | 31 | 71 | No food given since June 15. Fasting continued. |
| June 19 | Simple obstruction of ileum. | | | |
| June 20 | 38.7 | | 69 | Recovery satisfactory. |
| " 21 | 37.5 | 50 | 49 | Dull. Acts sick. Some feces. |
| " 22 | 37.0 | 74 | 37 | Very dull. Intoxication develops rapidly. Vomiting and diarrhea during day. Dead next morning. |

### TABLE II.

*Dog 18–138. Simple Obstruction of the Ileum.*

$$\textit{Ratio: } \frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}.$$

| Date. | Hour. | Urea injected. | Urea excreted per hr. | Blood urea per 100 cc. | Ratio. | Remarks. |
|---|---|---|---|---|---|---|
| *1918* | | *gm.* | *gm.* | *mg.* | | |
| June 18, p.m. | 1 | 20 | 1.89 | 123 | 15.3 | Control. |
| | 2 | | 1.61 | 106 | 15.1 | Marked diuresis. |
| | 3 | | 0.92 | 91 | 11.6 | |
| June 19 | Simple obstruction of ileum. | | | | | |
| June 22, p.m. | 1 | 20 | 0.64 | 212 | 3.0 | Intoxication of intense grade. |
| | 2 | | 0.40 | 164 | 2.4 | Urine not much increased by injection of urea. |
| | 3 | | 0.35 | 146 | 2.4 | |

*Dog 18–138 (Tables I and II).*—Long haired mongrel, female; weight 40.5 pounds.

June 18, 1918. After 3 days fast 20 gm. of urea dissolved in 118 cc. of distilled water were injected intravenously. Phenolsulfonephthalein was given intramuscularly at the same time. The simple urea ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

was then determined for each of the following 3 hours (Table II).

June 19. A simple obstruction was made 10 cm. above the ileocecal valve.

June 20. Dog was quiet but did not act sick. Small semisolid stool. Temperature 39.5°C.

June 21. Somewhat dull. Appears sick.

June 22. Moderate intoxication in forenoon. More marked in late afternoon. Pulse weak. Vomiting bile-stained mucus. Slight diarrhea late in day. Temperature 38.9°C. Died during the night.

*Autopsy.*—Lungs and heart normal. Peritoneum clean and glistening. Small intestine distended with gas and a thin, slimy, slate-colored fluid which had a putrid odor. Portions of the mucosa were engorged and velvety. A few petechial hemorrhages were seen in it. Stomach contained slight amount of bile-stained mucus. Liver and spleen moderately engorged with unclotted blood. Kidneys normal in appearance both grossly and microscopically. Remaining viscera likewise negative.

The protocol of Dog 18–138 together with Table I shows the typical acute reaction following a simple uncomplicated obstruction of the small intestine, although death does not ordinarily occur in such a short time. The animal usually recovers from the immediate effects of the operation and may remain active and bright for several days and in certain cases many days. Then rather abruptly, the symptoms of intoxication, namely dullness, temperature reaction, weakness of the pulse, vomiting, and prostration, appear. The dog may remain in this condition for several days before death or may show a rather precipitous decline and die during the night following the first appearance of these symptoms. In either case the blood urea may soar to twice or three times its normal value just before death, rising gradually from day to day in the cases in which the symptoms appear early and very rapidly in the instances of sudden intoxication and death.

Table I shows a gradual increase in the blood urea from day to day

and a corresponding gradual diminution in the percentage of phenolsulfonephthalein eliminated per 2 hours.

In Table II are presented data regarding the effect of the acute intoxication upon the urea-excreting power of the kidneys as measured by the urea ratio. It will be seen from these figures that the excretory function as far as urea is concerned is far below normal. The ratio of the urea excreted per hour to the urea per 100 cc. of blood was lowered from an average normal value of 14 before the obstruction was made to an average value of 2.6 during the height of the intoxication on the day preceding the death of the animal. The decrease in this ratio is, therefore, much more striking than that in the percentage of dye eliminated.

TABLE III.

*Dog 19–17. Simple Obstruction of the Ileum. Blood Urea. Phenolsulfonephthalein Elimination.*

| Date. | Weight. | Blood urea per 100 cc. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|
| *1918* | *lbs.* | *mg.* | *per cent* | |
| Aug. 8 | 30.3 | 28 | 70 | Dog fasted 3 previous days. |
| " 9 | 30.2 | 27 | 72 | |
| Aug. 10 | Simple obstruction just above ileocecal valve. | | | |
| Aug. 11 | 29.0 | 24 | 67 | Light attack of distemper. Recovery from operation satisfactory. |
| " 12 | 28.5 | 41 | 63 | Distemper worse. Intoxication definite in p.m. |
| " 13 | 27.3 | 97 | Mere trace. | 220 cc. of $\frac{M}{6}$ salt solution given intravenously soon after dye injection. Vomits; diarrhea. Killed. |

*Dog 19–17* (*Table III*).—Thin, long haired mongrel; weight 30.5 pounds.

Aug. 10, 1918. Simple obstruction made just above the ileocecal valve.

Aug. 11. Dog quiet. Shows slight distemper. Pulse and temperature normal.

Aug. 12. Weaker. Distemper somewhat worse. Laparotomy wound in good condition.

Aug. 13. Very dull. Vomits bile-stained mucus. Moderate diarrhea. Temperature 39.7°C. Killed at 10.40 a.m.

*Autopsy.*—Heart normal. Lungs show a few hyperemic areas over the surfaces and a reddening of the bronchial mucous membrane, but no definite pneu-

monia. The liver, spleen, and kidneys show moderate engorgement of blood. The intestine above the point of obstruction is moderately distended with slimy, putrid yellowish green fluid and gas. Its mucosa is hyperemic. The peritoneum is quite free and has its normal sheen.

*Microscopic Examination.*—Kidneys show a very slight degree of cloudy swelling. Otherwise entirely normal. Other organs likewise normal except for slight congestion of the smaller blood vessels.

The data given in the protocol of Dog 19–17 and in Table III serve to illustrate the second type of reaction to acute experimental obstruction; namely, that in which the symptoms of intoxication, including an increase in the blood urea, appear comparatively suddenly only a short time before death. The percentage of the test dye eliminated in this case was not decreased below the normal level until the last day when the most marked drop was recorded. So little dye was eliminated, in fact, that it could not be accurately estimated. Reference to the table shows that the rise in the blood urea was equally abrupt.

In this particular instance the resistance of the animal to the agent causing the intoxication was doubtless much lowered by the accompanying attack of distemper (*Bacillus bronchisepticus*) which had greatly weakened the dog before the reaction to the obstruction occurred. That an important part of the depression in the renal function was not due to the distemper may be inferred from the figures given in Table XII which show the absence of any definite impairment of kidney activity as measured by the ability to excrete urea and phenolsulfonephthalein in an uncomplicated case of distemper much more advanced than in this experiment.

*Dog 19–19 (Tables IV and V).*—Medium sized, short haired mongrel; weight 22.5 pounds.

Aug. 21, 1918. Simple obstruction made 10 cm. above ileocecal valve.

Aug. 22. Dog very active and bright. A small amount of milk and cracker meal eaten.

Aug. 23. Active and bright. No food given. Temperature 38.6°C. Pulse normal. Vomits.

Aug. 24. Slightly dull and weak. Pulse slightly weak. Temperature normal.

Aug. 25. Dog very dull. Vomits bile-stained mucus. Moderate diarrhea, late in day. Temperature 39.1°C. Pulse weak. Died during the night.

TABLE IV.

*Dog 19–19. Simple Obstruction of the Ileum. Blood Urea. Phenolsulfonephthalein Elimination.*

| Date. | Weight. | Blood urea per 100 cc. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|
| *1918* | *lbs.* | *mg.* | *per cent* | |
| Aug. 18 | 20.4 | 28 | 69 | No food previous 2 days. |
| " 19 | 20.4 | 27 | 68 | |
| " 20 | 20.2 | 29 | 70 | |
| Aug. 21 | Simple obstruction 10 cm. above ileocecal valve. | | | |
| Aug. 22 | 20.0 | 28 | 69 | Recovery from operation. |
| " 23 | 19.4 | 36 | 53 | A few drops of urine lost. |
| " 24 | 19.0 | 38 | 66 | Slightly dull. |
| " 25 | 18.4 | 93 | 32 | Vomits bile-stained mucus. Fluid feces. Intoxication. |

TABLE V.

*Dog 19–19. Simple Obstruction of the Ileum.*

$$\textit{Ratio: } \frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}.$$

| Date. | Hour. | Urea injected. | Urea excreted per hr. | Blood urea per 100 cc. | Ratio. | Remarks. |
|---|---|---|---|---|---|---|
| *1918* | | *gm.* | *gm.* | *mg.* | | |
| Aug. 18 | 8.15– 9.15 | | 0.10 | 28 | 3.5 | No food since Aug. 13. |
| | 9.30–11.30 | 10 | 1.046 | 91 | 11.5 | |
| Aug. 21 | Simple obstruction 10 cm. above ileocecal valve. | | | | | |
| Aug. 25 | 1.45– 2.45 | | 0.247 | 93 | 2.6 | Dog very sick. |
| | 3–5 | 10 | 0.734 | 165 | 4.4 | |

*Autopsy.*—Heart and lungs normal. Liver, spleen, kidneys, and mucosa of small intestine above obstruction moderately engorged with blood. Small intestine above obstruction distended with the typical slimy, putrid fluid and gas. No peritonitis.

*Microscopic Examination.*—Kidneys normal except for slight amount of congestion. Other organs also slightly congested.

The experiment presented in Tables IV and V illustrates the type of reaction in which the development of the intoxication is relatively precipitous, ending in death within 24 to 30 hours. The suddenness of the rise in blood urea and the fall in the percentage of test dye eliminated in the standard time is almost identical with that observed in the previous experiment (Table III). Dog 19–19 had no distemper and lived 1 day longer than Dog 19–17. This experiment serves as an additional check on the previous experiment which was complicated by the presence of distemper. The decrease in the urea ratio is rather striking here as in the first experiment.

TABLE VI.

*Dog 18–119. Simple Obstruction of the Ileum. Blood Urea. Phenolsulfonephthalein Elimination.*

| Date. | Weight. | Blood urea per 100 cc. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|
| *1918* | *lbs.* | *mg.* | *per cent* | |
| Sept. 4 | 23.8 | 28 | 76 | Fasted 3 previous days. |
| Sept. 5 | Simple obstruction of ileum. | | | |
| Sept. 6 | 23.4 | 27 | 80 | Recovery from operation satisfactory. |
| " 7 | 23.2 | 22 | 72 | Dog bright and active. |
| " 8 | 22.7 | 10 | 67 | |
| " 9–18 | 22–19 | 23–37 | 60–67 | Dog apparently normal during this period. ½ pint of milk given on Sept. 18. |
| " 19 | 18.4 | 33 | 66 | Showed first signs of intoxication. Dull. Vomited small amount. Temperature 39°C. |
| " 20 | 17.7 | 57 | 51 | Weak and dull. Vomited more after eating small amount of milk. Pulse slightly weak. |
| " 21 | 17.4 | 81 | 48 | Extremely dull and apathetic. Vomited. No diarrhea. Temperature 39.5°C. |
| " 22 | 17.0 | 124<br>140 | 22 | Extremely dull and weak. Vomited. Tenesmus. Late in p.m. Temperature 37.4°C. Died during night. |

*Dog 18–119 (Tables VI and VII).*—Small spaniel; weight 23 pounds. Has been employed several times previously for studies on proteose intoxication which will be reported in the following communication.

Sept. 5, 1918. Dog was bright and active. Temperature 39.1°C.

TABLE VII.

*Dog 18–119. Simple Obstruction of the Ileum.*

$$\textit{Ratio: } \frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}.$$

| Date. | Hour. | Urea injected. | Urea excreted per hr. | Blood urea per 100 cc. | Ratio. | Remarks. |
|---|---|---|---|---|---|---|
| *1918* | | *gm.* | *gm.* | *mg.* | | |
| Sept. 4 | 8.20– 9.20 | | 0.123 | 28 | 4.4 | |
| | 9.30–11.30 | 10 | 0.917 | 71 | 12.9 | Moderate diuresis. |
| Sept. 5 | Simple obstruction of the ileum. | | | | | |
| Sept. 10 | 12.40– 1.40 | | 0.119 | 28 | 4.2 | Dog quiet after operation. |
| | 1.50– 3.50 | 10 | 0.798 | 80 | 9.9 | Moderate diuresis. |
| " 12 | 8–9 | | 0.102 | 30 | 3.4 | No food given. |
| | 9.10–11.10 | 10 | 0.760 | 89 | 8.6 | Mild diuresis. |
| " 19 | 12.50– 1.50 | | 0.121 | 33 | 3.6 | Small amount of milk ingested on previous day. |
| | 2–4 | 10 | 0.613 | 112 | 5.5 | Slight diuresis. |
| " 22 | 10.20–11.20 | | 0.166 | 81 | 2.0 | Dog very sick. |
| | 11.30– 1.30 | 10 | 0.680 | 210 | 3.4 | No diuresis. Extra fluid given. |

Sept. 7. Still bright. Temperature 38.9°C.

Sept. 8. Slightly dull. Pulse slightly weak. Temperature 38.9°C.

Sept. 9 to 18. Bright but quiet. Pulse and temperature normal.

Sept. 18. Given ½ pint of whole milk containing lactose by stomach tube. Most of milk vomited.

Sept. 19. Acts dull. Vomits curdled milk and bile-stained mucus. Small mucus-coated stool. Temperature 39°C. Pulse slightly weak.

Sept. 20. Somewhat weaker and duller than on previous day. Vomits bile and watery mucus. Pulse weak.

Sept. 21. Extremely dull and apathetic. Vomits small quantity of mucus. No diarrhea. Temperature 39.5°C.

Sept. 22. Extremely dull and sick. Continues to vomit. Small hard mucus-coated stool. Pulse weak. Temperature at 6 p.m. 37.4°C. Died during the night. Vomitus and fluid feces in cage.

*Autopsy.*—Showed considerable emaciation. Heart and lungs normal. Peritoneal lining normal. Liver, spleen, and intestinal mucosa above point of obstruction are moderately hyperemic. Small intestine greatly distended with foul slimy fluid. Kidneys normal except for a slight grade of chronic pyelitis.

*Microscopic Examination.*—Moderate grade of chronic pyelitis. Remainder of kidney appears normal.

The protocol of Dog 18–119 presents a marked contrast to those given above in the length of time required for the intoxication to develop. The reason for this striking difference is not apparent from the data given unless we assume the possibility that a low grade tolerance has developed in the present case as a result of the previous injections of toxic proteose, in accordance with suggestions made in previous reports (17).

Table VI shows a relatively long period after the formation of the obstruction during which the blood urea and the elimination of phthalein remained normal. Following the ingestion of a very small amount of milk, most of which was vomited, the animal began to show symptoms of intoxication which continued to grow more intense until death 3 days later. As with Dog 18–138 (Tables I and II) there was found a gradual increase in the urea of the blood with a corresponding decrease in the amount of phthalein excreted.

Table VII shows an interesting change in the ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

both with and without the injection of urea. The difference between the normal value of the ratio before the obstruction and on the last day before death is very marked, especially when urea was injected. The decrease in the ratio appeared sooner than the fall in the phthalein output and was much more marked, as will be seen from inspection of both tables.

At autopsy a moderate grade of chronic pyelitis was found to be present although the remainder of the kidney both microscopically and grossly appeared normal. To what extent this condition was responsible for the subnormal activity of the kidneys cannot be said. However, since the process was found to be one of long standing while the impairment of function as measured by the tests was limited to the last 3 days, in which respect it corresponds very well with the previously described experiments, it seems probable that the impairment was due to the intoxication associated with the obstruction.

TABLE VIII.

*Dog 19–55. Closed Loop of the Ileum. Acute and Chronic Intoxication. Blood Urea. Phenolsulfonephthalein Elimination.*

| Date. | Weight. | Blood urea per 100 cc. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|
| *1918* | *lbs.* | *mg.* | *per cent* | |
| Oct. 18–21 | 29.4 | 28–33 | 78–82 | Oct. 19. Chloride excretion determined. |
| " 21 | 29.0 | 36 | 65 | No food eaten. Drinks considerable water. |
| " 22 | 28.2 | 46 | 65 | Refuses food. Very dull. |
| " 23 | 28.2 | 56 | 24 | Dog very sick. Vomits. Diarrhea. Febrile reaction. |
| " 24 | 27.5 | 38 | 50 | Still dull but brighter than on previous day. |
| " 25 | 27.2 | 44 | | Chloride excretion determined for 24 hrs. Dull. Refuses food. |
| " 26 | 27.2 | | 54 | Slightly improved. |
| " 27 | 27.0 | 33 | 55 | Eats little food. Shows improvement. |
| Oct. 28–Dec. 3 | 24.5–26.5 | 28–40 | 55–67 | Nov. 6. Chloride excretion determined. Dog practically normal during period |
| Dec. 4 | 26.4 | 45–78 | 34 | 47 cc. of proteose solution injected intravenously. Mild reaction. |
| " 5 | 24.2 | | 54 | Recovered. |
| " 9 | 23.5 | 54 | 66 | Small dose of x-rays given. |
| " 10 | 23.2 | 44 | 65 | Dog clinically intoxicated. |
| " 11 | 23.0 | 30 | 57 | Slightly dull. Fluid feces. Vomited. |
| " 12 | 22.7 | 38 | 57 | Condition the same. |
| " 13 | 22.5 | 58 | 54 | Killed. Autopsy. |

TABLE IX.

*Dog 19–55. Closed Loop of the Ileum. Acute and Chronic Intoxication. Excretion of Injected Sodium Chloride.*

| Date. | Sodium chloride injected. | Chloride excretion over 24 hr. period. | | | | | | Remarks. |
|---|---|---|---|---|---|---|---|---|
| | | 1st 2 hrs. | 2nd 2 hrs. | 3rd 2 hrs. | 4th 2 hrs. | Remainder of 24 hrs. | Total 24 hrs. | |
| *1918* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | |
| Oct. 19 | 6 | 1.88 | 1.29 | 0.84 | 0.61 | 1.96 | 6.58 | 2 days after loop operation—control. Dog bright and active. |
| " 25 | 6 | 1.02 | 0.58 | 0.41 | 0.32 | 0.83 | 3.16 | Dog dull. Vomited. No food on previous day, or during present 24 hrs. |
| Nov. 6 | 6 | 1.89 | 1.42 | 0.94 | 0.62 | 2.69 | 7.56 | Dog bright and active. Mixed diet given on previous day and during experiment by error. |

*Dog 19–55 (Tables VIII and IX).*—Tall, thin setter, female; weight 29 pounds.

Oct. 17, 1918. Loop of ileum 30 cm. in length isolated and an anastomosis made to reestablish continuity of small intestine.

Oct. 18 to 21. Dog bright and active. Pulse and temperature normal.

Oct. 21. Slightly dull. Vomits food and mucus. Passes soft feces. Pulse normal. Temperature 38.9°C.

Oct. 22. Dull. Vomits small amount of mucus.

Oct. 23. Much more dull. Acts sick. Temperature 38.8°C. Vomits bile-stained watery mucus. Passes soft and semifluid feces.

Oct. 24. Still dull but slightly brighter than on previous day. Vomits small amount of foamy, bile-stained mucus. No diarrhea. Pulse and temperature about normal.

Oct. 25. Dog dull and weak. Vomits. Passed soft feces.

Oct. 26. Quiet but somewhat improved. No vomiting or diarrhea. Pulse and temperature normal.

Oct. 27. Vomited small amount. Fluid feces.

Oct. 28 to Dec. 3. Dog was quiet and acted weak but was moderately bright. Ate a small amount of food occasionally. Weight varied from day to day between 24.5 and 26.5 pounds. Blood urea varied from 16 to 40 mg. per 100 cc., and 2 hour phthalein elimination varied between 55 and 67 per cent.

Dec. 3. Slight distension of abdomen with faint peristaltic waves in loop observed. Dog slightly dull.

Dec. 4. 1.75 cc. of toxic proteose solution per pound of body weight given intravenously; this produced a mild grade of intoxication. Recovery from this was comparatively rapid.

Dec. 9. Distension of abdomen somewhat increased and peristalsis more distinct. At 5.30 p.m. given 190 milliampere minutes x-rays over abdomen in three doses.[1]

Dec. 10. Dog shows greater weakness and dullness than on previous day. Vomited bile-stained mucus and food. Moderate diarrhea.

Dec. 11. Brighter but very weak and quiet.

Dec. 12. Greater dullness. Pulse weak.

Dec. 13. Very dull and weak. Vomited; diarrhea. 11.30 a.m. Killed.

*Autopsy.*—Heart and lungs negative. Liver and spleen showed a moderate degree of atrophy. Pancreas normal. Kidneys normal except for pallor of the inner cortical zone due to the deposition of fat. Peritoneum clean and glistening. The isolated loop of ileum enormously distended with semifluid, slimy, greenish gray material with a fleshy, slightly putrid odor. The muscular coat was greatly hypertrophied. The mucosa of the loop was smooth and velvety in appearance with no visible signs of ulceration or necrosis.

*Microscopic Examination.*—Kidneys normal.

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[1] Detailed statement to be given in a subsequent report.

Table VIII with the protocol of Dog 19–55 presents some interesting facts. A 30 cm. closed loop of the lower portion of the ileum was isolated in the manner described elsewhere (17) by making two sections of the intestine, turning in the cut ends, and reestablishing the alimentary canal by a simple lateral anastomosis between the remaining portions. The animal recovered satisfactorily from the immediate effects of the operation and remained active and bright until the 4th day when symptoms of intoxication began to appear.

These symptoms became gradually more marked until the 6th day, after which they gradually decreased in intensity until recovery was apparently complete on the 8th day. The cause of this reaction was possibly not the presence of the isolated closed loop but a temporary stasis above the point of anastomosis, since the loop itself showed no enlargement or abnormal peristalsis typical of a distended loop. However, it cannot be denied that this may have been a loop intoxication followed by improvement due to established tolerance.

During this period the blood urea increased considerably above normal. The phthalein elimination showed the usual downward movement on the day of the most intense intoxication and remained slightly below normal as long as the symptoms of intoxication lasted. The blood urea likewise remained above the normal level for a fasting dog until recovery.

Table IX shows that the capacity of the kidneys to eliminate injected sodium chloride was also lowered to a moderate degree at this time and then returned to normal upon recovery.

From this date the dog remained practically normal for over a month, taking food daily and living without apparent inconvenience from the isolated loop. On December 4, 1.75 cc. of toxic proteose solution per pound of body weight were given intravenously. Only a mild grade of intoxication was produced, although, as will be seen from Table VIII, the blood urea on that day rose from 45 to 78 mg. per 100 cc. following the injection and the phthalein elimination dropped from 67 per cent on the preceding day to 34 per cent during the intoxication.

The intensity of the reaction due to this dose of the proteose preparation was appreciably less in this case than in the case of a normal dog (Dog 19–59). A slightly larger dose, in fact, *i.e.* 2 cc. per pound

of body weight, was shown to be the lethal dose for a normal dog (Dog 19–29) in another experiment. There appears, then, to have been a slightly increased resistance to proteose poisoning in the case of Dog 19–55 which suggests to us the possibility of this dog's having acquired a partial immunity to such intoxication following this subacute loop intoxication.

The x-ray treatment constitutes part of another experiment, which will be reported at another time.

TABLE X.

*Dog 19–42. Simple Obstruction of the Lower Portion of the Jejunum. Blood Urea. Phenolsulfonephthalein Elimination.*

| Date. | Weight. | Blood urea per 100 cc. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|
| *1918* | *lbs.* | *mg.* | *per cent* | |
| Oct. 10 | 27.0 | 29 | 70 | No food on previous day. |
| " 31 | 27.6 | 30 | 70 | Food given on previous day. |
| Nov. 1 | Simple obstruction at about middle of small intestine. | | | |
| Nov. 2 | 27.5 | 28 | 72 | Recovery from operation. |
| " 3 | 27.2 | 21 | 70 | Bright and active. |
| " 4 | 26.8 | 25 | 39 | Acts very dull. |
| " 5 | 26.4 | 39 | 68 | Drinks water but refuses food. Dull. |
| " 6 | 26.0 | 48 | | Chloride excretion determined (Table XI). Dull and weak. Vomits bile-stained mucus. |
| " 7 | 25.2 | 58 | 45 | Duller than on previous day. Vomiting increased. |
| " 8 | 24.0 | 50 | 32 | Condition unchanged. |
| " 9 | 23.2 | 64 | | Chloride excretion determined (Table XI). Very dull and sick. |

*Dog 19–42* (*Tables X and XI*).—Medium sized collie, adult female; weight 27 pounds.

Oct. 6, 1918. Dog fasted 3 previous days. Given 5 gm. of sodium chloride and 10 gm. of urea dissolved in 250 cc. of distilled water intravenously. Chloride excretion was then followed for the next 24 hours in three subperiods; *viz.*, 1st hour, next 2 hours, and remainder of 24 hour period.

Nov. 1. Simple obstruction produced at about the middle of small intestine in the usual way.

Nov. 2. Dog is quiet but not dull. Temperature 39°C.

TABLE XI.

*Dog 19–42. Simple Obstruction of the Lower Portion of the Jejunum. Excretion of Injected Sodium Chloride.*

| Date. | Sodium chloride injected. | Chloride excretion over 24 hr. period. | | | | Remarks. |
|---|---|---|---|---|---|---|
| | | 1st hr. | Next 2 hrs. | Remainder of 24 hrs. | Total 24 hrs. | |
| *1918* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | |
| Oct. 6 | 5.0 | 0.822 | 0.841 | 3.327 | 4.989 | Fasted 3 previous days. Active and bright. |
| Nov. 1 | Simple obstruction at middle of small intestine. | | | | | |
| Nov. 6 | 5.0 | 0.395 | 0.412 | 1.180 | 1.987 | |
| " 9 | 5.0 | 0.105 | 0.176 | 0.233 | 0.514 | Total period about 22 hrs. instead of 24. |

Nov. 3. Much brighter. Normal pulse and temperature.

Nov. 4. Acts very dull, lying down most of the time. Small amount of pus in wound. Pulse slightly weak. Temperature normal.

Nov. 5. Still dull. Drinks water but refuses food. Pulse slightly weak.

Nov. 6. Dull and weak. Vomited small quantity of bile-stained mucus. No diarrhea. Pulse weak.

Nov. 7. Condition unchanged.

Nov. 8. More dull. Vomiting increased. Clinically sick. Temperature 38.9°C.

Nov. 9. Very dull and sick. Abdomen moderately distended. Visible peristaltic waves. Vomited considerable clear, stringy, bile-stained mucus. Hard feces coated with mucus. Pulse slightly weak and irregular.

Nov. 10. Dog died at about 6 a.m. Fresh vomitus and fluid feces in cage.

*Autopsy.*—Body still warm. Rigor mortis incomplete. Wound showed slight amount of suppuration, but mostly healed. No peritonitis. Heart and lungs normal. Spleen and liver irregularly engorged with unclotted blood. Kidneys negative except for slight pallor of inner zone of cortex due to deposition of fat. Pancreas negative. Small intestine above point of obstruction greatly distended with typical semifluid, yellowish green material with a foul odor. The mucosa is irregularly hyperemic but is not ulcerated. The portion of intestine below the obstruction is contracted but has a normal appearance.

*Microscopic Examination.*—Kidneys entirely normal. Other organs likewise normal.

Table X (Dog 19–42) shows a gradual increase in the urea content of the blood and a corresponding decrease in the percentage of

phthalein eliminated from day to day following the production of a simple obstruction of the jejunum.

Table XI gives the rate of excretion of injected sodium chloride over a 24 hour period before the operation, on the 5th day afterwards, and again on the last day before the animal died. Inspection of the figures recorded on the various days shows a marked diminution in the quantity of salt eliminated. Between one-half and one-third only of the normal amount of total chlorides was excreted on the 5th day and only slightly less than one-tenth of the normal quantity appeared in the urine of the last day. The degree of retention of chlorides during the intoxication appears to be much greater, therefore, than that of urea and phenolsulfonephthalein.

In this case as in all the above with the exception of Dog 18–119 (Tables VI and VII) the kidneys appeared normal both grossly and microscopically, in spite of the terminal impairment of renal function.

TABLE XII.

*Dog 19–24. Distemper (B. bronchisepticus).*

*Ratio:* $\frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}$.

*Blood Urea. Phenolsulfonephthalein Elimination.*

| Date. | Hour. | Urea injected. | Urea excreted per hr. | Blood urea per 100 cc. | Ratio. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|---|---|---|
| *1918* | | *gm.* | *gm.* | *mg.* | | *per cent* | |
| Sept. 5 | 8–9 | | 0.131 | 29 | 4.6 | | |
| | 9.10–10.10 | 10 | 0.720 | 83 | 8.69 | 68 | Dog active and bright. Moderate diuresis after urea injection. |
| | 10.10–11.10 | | 0.659 | 81 | 8.16 | | |
| Sept. 8 | Develops distemper. | | | | | | |
| Sept. 19 | 8.50– 9.50 | | 0.174 | 41 | 4.2 | | |
| | 10–11 | 10 | 0.727 | 94 | 7.7 | 60 | Dog in moribund condition. Blood pressure extremely low. Moderate diuresis after urea injection. |
| | 11–12 | | 0.658 | 92 | 7.1 | | |

*Dog 19–24* (*Table XII*).—Large Scotch collie, female; weight 30.5 pounds.

Sept. 5, 1918. At the end of 3 days fast urea ratio determined after injection of 10 gm. of urea (Table XII). 2 hour phthalein elimination 68 per cent.

Sept. 8. Began to show signs of distemper (*B. bronchisepticus* infection). Acted slightly dull. Sneezed occasionally. Slight amount of exudate about nares and eyes.

Sept. 10. Frank distemper. Dull. Temperature high. Increased exudate about nares and conjunctivæ. Weight 27.5 pounds.

Sept. 18. Condition much worse. Very weak and apathetic. Pulse extremely weak. Lies in cage twitching and jerking. Nares and eyes covered with mucopurulent exudate. Weight 23.5 pounds. Phthalein elimination for 2 hours, 61 per cent. Blood urea 42 mg. per 100 cc.

Sept. 19. Dog moribund. Condition of previous day aggravated considerably. Dye elimination 60 per cent. Blood urea 41 mg. 3 p.m. Killed.

*Autopsy.*—Heart normal. Lungs show diffuse patches of hyperemia and edema. Bronchial passages very hyperemic. Bronchial exudate mucopurulent. Lymph nodes hypertrophied. Liver, spleen, kidneys, pancreas, and gastrointestinal tract normal.

*Microscopic Examination.*—Kidneys and liver show slight degree of cloudy swelling, but are otherwise normal. Lungs show engorgement and edema. Other organs normal.

The last experiment of the present series on a dog with uncomplicated distemper is included as a control for one of the foregoing numbers in particular (Table III), in which the animal developed a moderate case of distemper after the operation for the production of the obstruction. It serves for a check also on the other experiments because of the common factor of low blood pressure in both conditions, which, if not controlled, might invalidate any study on renal function. The severity of the infection was much more extreme in the present case than in that of Dog 19–17, and the fall in blood pressure was much greater than is often observed in the intoxication of intestinal obstruction except in the last hours after the animal has passed into a state of shock.

Table XII shows the non-influence of the most advanced stage of distemper upon the urea ratio and the phenolsulfonephthalein elimination. Although the blood pressure was extremely low during the distemper period, the urea ratio and the percentage of dye excreted were only very slightly below the normal. This fact justifies the assumption, therefore, that the marked retention of urea, phthalein,

and chlorides during the period of intoxication following obstruction of the small intestine is only in very small part, if at all, due to the factor of lowered blood pressure.

## DISCUSSION.

The experiments presented in the above tables definitely clear up the question set forth in the early part of this paper with reference to the efficiency of the kidneys during the intoxication of acute intestinal obstruction. The excretory function is decidedly impaired in this condition.

Individuals with intestinal obstruction show a heaping up of all non-protein nitrogenous substances in the blood. Urea is most conspicuous in this material. The kidney is evidently unable to secrete any of these nitrogenous substances with its normal facility. These substances are being formed with abnormal speed, so there is a great accumulation in the blood and tissues. The kidney in this condition reacts much like the kidney of chronic nephritis, although there is no anatomical injury and the kidney of intestinal obstruction is only temporarily insufficient. With relief of the obstruction and clinical recovery the kidney function returns to normal. This injury can be repaired easily and leaves no trace behind, in as far as modern histological methods can show.

The decrease in output of fluid in the urine is due in part to the loss of fluid by vomiting, but when fluids are given intravenously the lack of diuresis may well be explained by the injury of renal epithelium. It has long been known that certain proteoses inhibit the flow of urine, but the experiments which demonstrated this were performed before the day of kidney function tests.

That dogs with very severe distemper and bronchopneumonia show little if any drop in renal function is somewhat surprising. It is known that these animals at times show a high non-protein nitrogen, but never approaching the high figures of intestinal obstruction. The dogs also show a great rise in the basal urinary nitrogen excretion, indicating a considerable breaking down of body protein. These facts demonstrate a clear-cut difference between the intoxications of pneumonia and intestinal obstruction in dogs.

We wish to recall three important facts concerning the intoxication of intestinal obstruction: (1) There is a great increase in the elimination of urinary nitrogen, which is dependent upon the intoxication. (2) There is a great increase in the non-protein elements of the blood. These two facts indicate cell injury. (3) There is a decrease in kidney excretory function which is most clearly shown by the inability of the kidney to secrete the normal amounts of urea, sodium chloride, and phenolsulfonephthalein.

The last fact is most important in establishing beyond reasonable doubt the presence of some poison in the blood stream. These experiments taken together with those outlined in the next paper indicate again that the same poison is also present in the lumen of the obstructed intestine. Some may insist upon the isolation of some poison from the blood stream, but with our present methods that proof cannot be given. In fact the whole blood is non-toxic, but this is no proof that no poison exists. A lethal dose of proteose will disappear from the blood stream within 3 to 5 minutes and yet will work its fatal reaction which may require 6 to 8 hours or longer for its completion.

We believe in picturing this reaction in the kidneys as a part of the general cell protein injury which results from the presence of the obstructed intestine. The poison, let us say, acts directly upon the epithelium of the kidney and causes temporary paralysis or impairment of its secretory function. There is no histological evidence of any cell injury, but we realize that function may be impaired without any definite change in structure. Repair of this injury may be effected within 24 to 48 hours after clinical recovery from the intoxication.

In the treatment of this condition no physician can afford to ignore this established fact that a definite impairment of kidney function develops as a part of the intoxication of intestinal obstruction. The two conditions usually parallel each other closely. The degree of intoxication which may develop in ileus is sometimes hard to evaluate clinically. We suggest that the non-protein nitrogen or urea nitrogen of the blood, as well as the renal function, may give warning of a grave intoxication which may be masked clinically. We are aware that ileus may persist with stormy symptoms for many days without really grave intoxication. Again, the condition may appear to be mild

clinically yet associated with high blood urea and a low renal function. In the last instance there should be no doubt of a serious intoxication and the necessity of urgent measures.

## SUMMARY AND CONCLUSIONS.

Associated with the intoxication of intestinal obstruction there exists a definite impairment of the excretory function of the kidneys.

The degree of functional depression corresponds roughly with the intensity of the clinical intoxication.

The decrease in the urea ratio and in the capacity of the kidneys to excrete sodium chloride is more marked than is the percentage decrease of phenolsulfonephthalein elimination.

The great increase in the non-protein nitrogen of the blood usually observed in acute intestinal obstruction, which has hitherto been explained as being due entirely to an increased rate of protein catabolism, is due in part to retention of the products released from the injured cell protein.

It is probable that the impaired renal function is due to direct action of the toxic substances upon the renal epithelium.

The actual demonstration of this renal injury is perhaps the strongest evidence so far obtained to prove the presence of an actual toxic substance in the blood during intestinal obstruction.

This obscure disability of the kidneys during the height of the intoxication of acute ileus should always be considered in the clinical management of this condition. It may also serve as a guide to indicate the degree of intoxication.

## BIBLIOGRAPHY.

1. Hartwell, J. A., and Hoguet, J. P., *Am. J. Med. Sc.*, 1912, cxliii, 357.
2. Hartwell, J. A., Hoguet, J. P., and Beekman, F., *Arch. Int. Med.*, 1914, xiii, 701.
3. Tileston, W., and Comfort, C. W., Jr., *Arch. Int. Med.*, 1914, xiv, 620.
4. Cooke, J. V., Rodenbaugh, F. H., and Whipple, G. H., *J. Exp. Med.*, 1916, xxiii, 717.
5. Myers, V. C., and Lough, W. G., *Arch. Int. Med.*, 1915, xvi, 536.
6. Whipple, G. H., and Van Slyke, D. D., *J. Exp. Med.*, 1918, xxviii, 213.
7. Christian, H. A., *J. Urol.*, 1917, i, 320.

8. Austin, J. H., and Eisenbrey, A. B., *J. Exp. Med.*, 1911, xiv, 366.
9. Rowntree, L. G., Marshall, E. K., Jr., and Baetjer, W. A., *Arch. Int. Med.*, 1915, xv, 543.
10. Rowntree, L. G., and Geraghty, J. T., *J. Pharmacol. and Exp. Therap.*, 1909–10, i, 579.
11. Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1912, ix, 284.
12. Addis, T., and Watanabe, C. K., *J. Biol. Chem.*, 1916–17, xxviii, 251.
13. Watanabe, C. K., Oliver, J., and Addis, T., *J. Exp. Med.*, 1918, xxviii, 359.
14. Van Slyke, D. D., and Cullen, G. E., *J. Am. Med. Assn.*, 1914, lxii, 1558.
15. Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, xix, 141.
16. McLean, F. C., and Van Slyke, D. D., *J. Biol. Chem.*, 1915, xxi, 361.
17. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *J. Exp. Med.*, 1914, xix, 144.

# II. RENAL FUNCTION INFLUENCED BY PROTEOSE INTOXICATION.

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In the preceding paper (1) we reported a number of experiments which showed that the excretory function of the kidneys is definitely impaired during the intoxication of intestinal obstruction. Since most of the animals employed in that study died late during the night, the maximum degree of impairment was not always ascertained by the tests made on the previous day. This fact together with a number of other considerations made it highly desirable that the study should be continued under somewhat different conditions in which the reaction could be better controlled.

It has been shown (2–6) that the intravenous injection of the toxic proteose material isolated from the contents of the obstructed small intestine produces symptoms closely resembling, if not identical with, those characterizing the intoxication of acute ileus. Therefore, we decided to employ this more simple method of inducing the intoxication for a supplementary study of the renal function. In this way the time and the intensity of the reaction can be regulated at will for various experiments. Such a study should at the same time supply further evidence for or against the contention that the intoxication of acute intestinal obstruction is a proteose intoxication as has been suggested by Whipple, Stone, and Bernheim (2–5) and Whipple, Rodenbaugh, and Kilgore (6).

It has already been satisfactorily determined that no anatomical lesion of the kidneys is produced by the parenteral injection of the toxic proteose material. No histological alterations have ever been observed in the kidneys of normal dogs given fatal doses in this laboratory. In the anatomical laboratory of this University, furthermore, rats were stained *intra vitam* with Niagara blue, a relatively non-toxic vital dye, after having received intraperitoneally a series of large sublethal doses of toxic proteose. Although every grade of intoxication was produced in these animals, no definite changes in the structure of the kidneys that could be associated with the intoxication were demonstrated even by this delicate method of detecting cell injury.[1] However, this does not necessarily mean that there may not have been even a profound temporary impairment of renal

[1] McQuarrie, I., unpublished experiments.

function, since it was shown in the preceding paper that a definite impairment exists during the intoxication of intestinal obstruction in spite of the absence of anatomically demonstrable changes in the kidney.

The rapid rise in the non-protein nitrogen of the blood often to a very high level, observed (7) to follow the injection of toxic proteose in a fasting animal suggests inefficient activity on the part of the kidneys or unusually rapid destruction of body protein. That there is a greatly increased protein catabolism has been satisfactorily demonstrated by experiments (8) which showed a marked increase in the total urinary nitrogen excretion to follow the injection of a large dose of toxic proteose. A curious fact about this increase in total urinary nitrogen is that the apex of the curve of excretion occurs almost invariably during the second 24 hours after the injection of the poison, while the blood non-protein nitrogen reaches its maximum level on the 1st day shortly after the clinical signs of intoxication are most manifest. No entirely satisfactory explanation for this peculiar delay has been offered, although it suggests either a partial delay in the process of tissue disintegration or a transient impairment of kidney function.

## *Methods.*

Dogs, chiefly females, were used here as in the previous experiments because they react characteristically to proteose intoxication and are easily bled and catheterized. Only healthy young adult animals were selected after renal function tests showed them to possess normal kidneys.

The various methods employed for measuring the kidney function in the experiments described in the preceding communication were applied in the present work also; namely, (1) that of determining the urea-excreting power of the kidneys; (2) that of determining the rate of excretion of injected sodium chloride; (3) the phenolsulfonephthalein elimination method of Rowntree and Geraghty (9, 10).

The last two methods were employed here in exactly the same manner as in the previous work. The first method, that of measuring the urea-excreting capacity of the kidneys, was modified somewhat in the experiments to be described here. In addition to determining the ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

after the injection of urea intravenously, we determined it without the urea administration in a number of instances. In still other

cases the rate of excretion of injected urea over a 5 hour period was determined before and again after the injection of the toxic proteose preparation without reference to the blood urea. Wherever the ratio was determined, the blood sample was taken as nearly in the middle of the hour as possible.

The data obtained on each dog with regard to the renal function at the time of selection serve as a control for the later experiments on that particular animal, since the same general conditions obtained in both periods.

After this preliminary examination the dog was given a dose of the toxic material isolated from the contents of an obstructed intestine. From 1 to 3 hours after this injection the different renal function tests were applied as in the control period.

The poisonous material used in this study for producing the intoxication is isolated from the contents of the experimentally obstructed intestine of the dog and from human autopsy material in the following manner. The crude semifluid product is centrifugalized at high speed until all the coarser particles have been thrown to the bottom of the tube. The toxic fraction is then precipitated from the supernatant fluid by the addition of five volumes of 95 per cent alcohol. The flocculent alcoholic precipitate is separated by centrifugalization from the alcoholic mixture, after which it is redissolved in a volume of distilled water equal to three times that of the original obstruction fluid material. This solution which always contains a considerable quantity of albuminous material is made faintly acid with acetic acid and brought to the boiling point in a water bath. The precipitated proteins are filtered off, leaving a clear, slightly opalescent solution containing the toxic proteoses.

As has been pointed out elsewhere by Whipple and Van Slyke (11), the chemistry of the proteose group of substances is not at all clearly understood and what is spoken of in the present paper as a "proteose solution" is simply an aqueous solution of the alcoholic precipitate obtained in the manner described above, from which the albuminous material has been removed. Nevertheless, the unusual substance or group of substances isolated in this way from the mixture contained in the lumen of the obstructed small intestine gives the symptom complex characteristic of acute obstruction when injected

intravenously in the dog and, therefore, serves our present purposes very well.

A few other proteoses from various sources have also been used to supplement the experiments performed with the poisonous material already described. The object of including them in the present study was to ascertain to what extent the impairment of renal function is a property of all proteoses or to see if any other proteoses behave in this way.

Proteoses were obtained from the following sources: (1) commercial Witte's "Peptone," which consists chiefly of proteoses; (2) pepsin digest of liver (Martin's broth); (3) pepsin digest of sterile muscle tissue; (4) pepsin digest of muscle tissue accidentally contaminated with proteolytic bacteria, some of which were found to be anaerobes; (5) sterile muscle tissue digested by three different types of proteolytic anaerobic bacteria separately in pure culture. The three organisms used for the last digestion method were *Bacillus histolyticus*, *Bacillus sporogenes*, and *Bacillus bifermentans*.[2]

Material from each of the several protein digests was injected from time to time at different stages of the digestion, beginning on the 1st day in nearly all cases and continuing as long as 3 weeks in some. Chemical tests for determining the approximate yield of proteoses were made from time to time. In all cases the supernatant fluid from the digests was first filtered to remove larger particles. The filtrate was then heated for 20 minutes over a boiling water bath for sterilization and again filtered before it was injected. In the only case in which marked symptoms of intoxication were produced by this preliminary injection, namely that of the digest accidentally contaminated with proteolytic bacteria, the toxic mixture was treated in a manner exactly like that described above for the isolation of the toxic proteose from intestinal obstruction material.

From $1\frac{1}{2}$ to 3 hours after the injection of the proteose preparation in each instance, the blood urea was determined and the phenolsulfonephthalein elimination test was performed.

[2] These three organisms were suggested to us by Dr. K. F. Meyer and furnished in pure culture by Mrs. H. H. Heller.

In the experiments which follow, the same general methods of administering test substances, of collecting blood and urine samples, and of quantitating the urea, chlorides, and phthalein were used as those described in the preceding communication with a few minor differences. For example, in one short series of experiments the dye was given intravenously and in another set subcutaneously instead of intramuscularly. It was thought that the use of a variety of routes for the dye might afford some evidence regarding the part played by absorption in the failure of the intoxicated animal to eliminate it promptly.

In order better to control the extrarenal factor of loss of fluid from the blood to the tissues and to the alimentary tract during the period of intoxication, comparatively large amounts of fluid were given either by stomach tube when vomiting was slight or intravenously when the animal vomited frequently. Isotonic saline or glucose solution and in a few cases acacia solution were employed for this purpose and in every instance more fluid was given during the period of intoxication than during the corresponding control period.

## EXPERIMENTAL OBSERVATIONS.

In most of the following tables several like experiments have been grouped together in the same table in order that they may be easily compared and allow a saving of space. It will be observed, too, that the various grades of intoxication produced are spoken of as "slight," "mild," "moderate," and "intense." There is no very well defined line of demarkation between these, but by a "slight" intoxication is meant a definite, though transient febrile reaction, nausea with a slight amount of vomiting of mucus which is not necessarily bile-stained, usually a slight amount of tenesmus without definite diarrhea, and always a slight dullness. A "mild" intoxication is a somewhat more noticeable upset in which the rectal temperature may reach 40°C. and there is occasional vomiting of foamy, stringy, usually bile-stained mucus some time during the first 2 hours with more marked tenesmus and a soft or semifluid stool and more definite dullness. By a "moderate" grade of intoxication is meant a reaction still more marked in which the temperature may rise above 41°C.

and remain above normal for a number of hours; the blood pressure falls slightly below normal; there is intermittent vomiting of bile-stained mucus over several hours and frequent passage of small, soft and then semifluid stools, and considerable tenesmus. The animal appears dull and somewhat weak for a number of hours and often remains slightly dull even on the following day. An "intense" reaction following the injection of a lethal or slightly sublethal dose of the toxic substance is usually one in which there is frequent vomiting over the first few hours and diarrhea usually lasting longer, lowering of blood pressure, an early rise in temperature far above the normal with a later fall to a subnormal level. As the intestines are emptied the character of the excreta changes from the hard normal stool to a soft and finally a semifluid consistency. When a large portion of this has been evacuated, flakes or casts of mucus and epithelium from the intestinal mucosa begin to appear. Following this there is often found a small amount of clear, watery mucus which in the fatal cases is usually blood-stained. At this stage the body temperature may be considerably below normal and the sphincter ani is relaxed, allowing the watery excreta to ooze out slowly. The animal may show great prostration just before this final collapse or may die in convulsions. While this is the usual picture when the intoxication is prolonged, a large dose of the poison may cause death within an hour or two with little or no vomiting or diarrhea. In the more severe grades of intoxication there is nearly always a rapid rise in the non-protein nitrogen of the blood which runs parallel in a general way with the intensity of the intoxication.

Table I represents four short experiments in which the rate of excretion of injected urea was determined before and again after the injection of a sublethal dose of toxic proteose from a human autopsy which produced in each case a moderate grade of intoxication. The dye was injected subcutaneously at the same time that the urea was given intravenously.

These experiments uniformly show a moderate decrease in the amount of both urea and phthalein eliminated after the proteose injection. With the dye it will be noted that the 1st hour excretion suffers proportionately a decidedly greater decrease below the normal than the 2nd hour. This is doubtless due in part to the greater

TABLE 1.

*Injection of Proteose. Excretion of Injected Urea and Phenolsulfonephthalein.*

| | Proteose injected. | Urea injected. | Urea excretion. | | | | | | | Phthalein elimination. | | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Fore-period of 1 hr. | Length of time after urea injection. | | | | | | | | | |
| | | | | 1st hr. | 2nd hr. | 3rd hr. | 4th hr. | 5th hr. | Total. | 1st hr. | 2nd hr. | Total for 2 hrs. | |
| *1918* | *cc.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *per cent* | *per cent* | *per cent* | |
| Dog 18–2, weight 49 lbs. | | | | | | | | | | | | | |
| Feb. 22 | 0 | 25 | 0.19 | 2.30 | 2.11 | 1.60 | 1.35 | 1.13 | 8.68 | 47 | 26 | 73 | Marked diuresis. |
| " 28 | 98 | 25 | 0.19 | 1.46 | 1.21 | 1.07 | 0.88 | 0.73 | 5.54 | 31 | 20 | 51 | Urea and dye injected 1 hr. 10 min. after proteose. Moderate intoxication. |
| Dog 18–3, weight 36 lbs. | | | | | | | | | | | | | |
| Feb. 20 | 0 | 18 | 0.17 | 1.92 | 1.58 | 1.33 | 1.20 | 1.05 | 7.25 | 47 | 30 | 77 | Marked diuresis. |
| Mar. 5 | 72 | 18 | 0.18 | 1.36 | 0.92 | 0.93 | 0.81 | 0.63 | 4.83 | 25 | 21 | 46 | Urea and dye injected 1½ hrs. after proteose. Intoxication only mild. |
| Dog 18–85, weight 19 lbs. | | | | | | | | | | | | | |
| Mar. 2 | 0 | 12 | 0.20 | 1.62 | 1.25 | 1.03 | 0.75 | 0.61 | 5.46 | 54 | 23 | 77 | Marked diuresis. |
| " 10 | 37 | 12 | 0.18 | 0.87 | 0.78 | 0.54 | 0.47 | 0.37 | 3.21 | 31 | 23 | 54 | Urea and dye injected 1¼ hrs. after proteose. Moderate intoxication. |
| Dog 17–227, weight 40 lbs. | | | | | | | | | | | | | |
| Mar. 19 | 0 | 15.5 | 0.17 | 1.56 | 1.20 | 1.08 | 0.94 | 0.81 | 5.76 | 47 | 25 | 72 | Considerable diuresis. |
| Apr. 1 | 80 | 15.5 | 0.16 | 1.26 | 1.07 | 0.50 | 0.25 | 0.20 | 3.44 | 28 | 21 | 49 | Urea and dye injected 1½ hrs. after proteose. Moderate intoxication. |

relative concentration of dye in the blood stream during the 2nd hour in the case of the intoxication period, but it may be due in part also to slower absorption of the dye under these circumstances.

Although not given in Table I the volume of urine excreted during some of the later hours of the period of intoxication was occasionally slightly greater than that of the corresponding hours of the control period, due perhaps, in some cases at least, to the increased amount of water taken by the dog after the most acute stage of the intoxication had passed. Yet, there is in these instances, also, a considerable decrease in the amount of urea excreted, which seems to show that extrarenal factors, such as a lessened availability of water to be secreted, alone are not responsible for the failure of the animal to eliminate the normal quantity of administered urea during the intoxication.

*Dog 18–2 (Table II).*—

*Autopsy.*—Performed immediately after death. Heart distended with dark, prune-colored blood which coagulates very slowly. Numerous subendocardial petechial hemorrhages in ventricles. Lungs normal. Liver and spleen intensely engorged. Kidneys negative except for marked congestion. Intestinal mucosa very intensely engorged with blood. Considerable blood in lumen of intestines.

*Microscopic Examination.*—Shows nothing except congestion of all the viscera including the kidneys.

Table II shows the effect of various grades of proteose intoxications upon the ratio:

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea in 100 cc. of blood}}$$

when no urea is administered and upon the elimination of phenolsulfonephthalein.

It will be seen from these experiments that there is a lowering of the urea ratio and an almost parallel reduction in the amount of dye eliminated, both of which correspond with the grade of intoxication. Although in the cases of less intense intoxication the absolute amount of urea excreted per hour is slightly increased as the blood urea rises, the increase does not keep pace with this rise and consequently the ratio falls. This indicates a deficiency in the response of the kidney to even a small load. As will be seen in the control periods of some

TABLE II.

*Injection of Proteose. Effect upon the Ratio* $\frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}$ *and the Elimination of Phenolsulfonephthalein. No Urea Injected.*

| | Condition of experiment. | Hour. | Urea excreted. | Blood urea per 100 cc. | Urea ratio. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|---|---|---|
| *1918* | | | *gm.* | *mg.* | | *per cent* | |
| Dog 18–2, weight 49 lbs. | | | | | | | |
| Apr. 20 | Control. | 9–10 | 0.124 | 28 | 4.43 | | 300 cc. of water by stomach tube ½ hr. before phthalein. |
| | | 10–11 | 0.105 | 25 | 4.20 | 74 | |
| | | 11–12 | 0.117 | 26 | 4.50 | | |
| " 29 | Intense proteose intoxication. | 11.30–12.30 | 0.062 | 44 | 1.41 | | 10.40 a.m. 96 cc. of proteose injected. 12.30 p.m. Dye injected. 300 cc. of water by tube 11.50 a.m., partly vomited. |
| | | 12.30– 1.30 | 0.052 | 48 | 1.08 | Mere trace. | 12.50 p.m. 200 cc. of $\frac{M}{6}$ salt solution intravenously. |
| | | 1.30– 2.30 | 0.043 | 52 | 0.82 | | Marked oliguria. Apr. 30. Dog died from the intoxication. Blood urea 90 mg. |
| Dog 18–119, weight 24 lbs. | | | | | | | |
| July 8 | Control. | 9.45–10.45 | 0.271 | 34 | 7.60 | 71* | 10.15 a.m. Dye injected. |
| | Mild intoxication. | 1–2 | 0.183 | 40 | 4.50 | 57* | 11 a.m. Proteose injected. 12.30 p.m. 250 cc. of water. 1.30 p.m. Dye injected. |

* Dye given intravenously—30 minute collection.

TABLE II—*Concluded.*

| | Condition of experiment. | Hour. | Urea excreted. | Blood urea per 100 cc. | Urea ratio. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|---|---|---|
| *1918* | | | *gm.* | *mg.* | | *per cent* | |
| July 20 | Control. | 9–10 | 0.122 | 27 | 4.43 | 70* | |
| | Moderate intoxication. | 12.30– 1.30 | 0.146 | 41 | 3.54 | | 10.20 a.m. 150 cc. of dilute proteose solution. |
| | | 2.32– 3.32 | 0.128 | 57 | 2.25 | 41* | 1.35 p.m. 200 cc. of 5% acacia solution intravenously. |
| | | 8–9 next day. | 0.146 | 43 | 3.4 | 58* | 2.20 p.m. Dye injected. 2nd sample of blood at end instead of middle of hr. as usual. Dog slightly dull next day. |
| " 24 | Control. | 10–11 | 0.145 | 36 | 4.0 | | |
| | Very slight intoxication. | 1–2 | 0.160 | 44 | 3.6 | | 12 m. 23 cc. of dilute proteose solution. |
| | | 2–3 | 0.200 | 38 | 5.2 | 62* | 3.15 p.m. Dye injected. |
| Dog 19–59, weight 49 lbs. | | | | | | | |
| July 10 | Control. | 10–11 | 0.234 | 38 | 6.14 | 72* | |
| | Moderate intoxication. | 3–4 | 0.239 | 67 | 3.47 | 50* | 11.30 a.m. 400 cc. of very dilute proteose. 4.15 p.m. Dye in 100 cc. of water. |

of the following experiments (Tables III and IV), the normal kidney responds to an increase in the blood urea by producing a rise in the ratio and never a fall, as in this case.

Dog 18–2 with an intense grade of intoxication shows even a decrease in the quantity of urea excreted per hour although the blood urea has increased to 100 per cent above its normal value in a fasting dog. The ratio has decreased from one-fifth to one-third of its value before the proteose injection. The amount of dye eliminated in this 3 hour period was too small for accurate estimation by the colori-

metric method. There was marked oliguria in spite of the fact that a large quantity of fluid was given intravenously and by mouth.

It will be noted that, although the dye was given intravenously in the majority of the experiments shown in the table, the percentage eliminated was decreased in these as in other cases and that the decrease corresponded with the grade of intoxication. This observation proves that the factor of delayed absorption of the dye does not account for the diminution in the quantity excreted during the intoxication.

*Dog 17–227 (Table III).—*

*Autopsy.*—Heart and lungs negative. Liver markedly congested. Spleen intensely engorged with blood. Large subcapsular hemorrhages bulge from surface. Kidneys greatly congested. Otherwise normal. Gastrointestinal tract negative except for most intense engorgement of blood capillaries in mucosa of jejunum, duodenum, and rectum with less marked congestion in other regions.

*Microscopic Examination.*—Kidneys normal except for congestion. Other viscera also congested.

Tables III and IV include four experiments in which the urea ratio and phenolsulfonephthalein elimination were determined before and again after injection of various doses of toxic proteose. In each case except that of Dog 18–59 a 1 hour control period preceded the injection of urea and in only one case, that of Dog 17–227, did this control hour come after the injection of the poison. The data obtained for the fore-hour in the larger control period can be taken as a baseline from which to estimate more accurately the increase in the urea excreted per hour and in the blood urea due to the injection of urea alone. A comparison of these values with those obtained after the proteose injection shows the changes due to the proteose alone.

The decrease in the ratio in these experiments is very striking and is considerably greater than that found in the experiments presented in Table II in which no urea was injected. The reduction in the rate of phthalein elimination corresponds more nearly with the decrease in the urea-excreting power as measured by the methods used in the previous experiments. The apparent lowering of renal efficiency here as in the previous experiments corresponds in a general way with the intensity of the intoxication.

TABLE III.

*Injection of Proteose. Effect upon the Ratio* $\frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}$ *and the Elimination of Phenolsulfonephthalein. Urea Injected.*

| | Proteose injected. | Urea injected. | Hour. | Urea excreted. | Blood urea per 100 cc. | Urea ratio. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| *1918* | *cc.* | *gm.* | | *gm.* | *mg.* | | *per cent* | |
| Dog 17–227, weight 40 lbs. | | | | | | | | |
| Apr. 22 | | | 9–10 | 0.144 | 30 | 4.8 | | |
| | | 20 | 10.15–11.15 | 1.726 | 121 | 14.2 | 71 | Marked diuresis for several hours. |
| | | | 11.15–12.15 | 1.526 | 104 | 14.6 | | |
| | | | 12.15– 1.15 | 0.917 | 86 | 10.5 | | |
| " 24 | | | 10–11 | 0.100 | 87 | 1.16 | | 9.15 a.m. Proteose injected. |
| | 114 | 20 | 11.20–12.20 | 0.333 | 230 | 1.45 | Mere trace in 3 hrs. | Intense intoxication. Marked oliguria in spite of much fluid intake and diuretics given. |
| | | | 1.20– 2.20 | Anuria. | 223 | 0 | | 2.25 p.m. Died in convulsions. |
| Dog 18–3, weight 36 lbs. | | | | | | | | |
| Apr. 14 | | | 8.40– 9.40 | 0.132 | 28 | 4.7 | | |
| | | 20 | 10–11 | 1.981 | 129 | 15.3 | | |
| | | | 11–12 | 1.491 | 121 | 12.3 | 75 | Marked diuresis. |
| | | | 12–1 | 1.024 | 96 | 11.3 | | |
| " 22 | | | 8.30– 9.30 | 0.120 | 26 | 4.6 | | 9.45 a.m. Proteose injected. 11.50 a.m. Urea and dye injected. Moderate grade of intoxication. |
| | 83 | 20 | 11.50–12.50 | 0.863 | 166 | 5.2 | | Mild diuresis. |
| | | | 12.50– 1.50 | 0.695 | 161 | 4.3 | 42 | |
| | | | 1.50– 2.50 | 0.537 | 136 | 3.9 | | |

TABLE IV.

*Injection of Proteose. Effect upon the Ratio* $\frac{\textit{Urea in 1 Hr.'s Urine}}{\textit{Urea in 100 Cc. of Blood}}$ *and the Elimination of Phenolsulfonephthalein. Urea Injected.*

| | Proteose injected. | Urea injected. | Hour. | Urea excreted. | Blood urea per 100 cc. | Urea ratio. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| *1918* | *cc.* | *gm.* | | *gm.* | *mg.* | | *per cent* | |
| Dog 18–59, weight 48 lbs. | | | | | | | | |
| Aug. 18 | | 18 | 9–10 | 1.887 | 135 | 13.9 | | |
| | | | 10–11 | 1.540 | 112 | 13.7 | 72 | Marked diuresis. |
| | | | 11–12 | 0.937 | 84 | 11.1 | | |
| " 24 | 90 | 18 | 1–2 | 0.997 | 195 | 5.1 | | 10.40 a.m. Proteose injected. |
| | | | 2–3 | 0.872 | 188 | 4.6 | 34 | Moderate grade of intoxication. Mild diuresis. 1.37 p.m. 200 cc. of 10% dextrose. |
| | | | 3–4 | 0.719 | 154 | 4.6 | | |
| Dog 18–85, weight 19 lbs. | | | | | | | | |
| Mar. 15 | | | 9–10 | 0.119 | 24 | 4.9 | | |
| | | 12 | 10.15–11.15 | 1.596 | 119 | 13.5 | 80 | Marked diuresis. |
| | | | 11.15–12.15 | 1.264 | 108 | 11.7 | | |
| " 20 | | | 8.45– 9.45 | 0.121 | 27 | 4.5 | | 10 a.m. Proteose injected. |
| | 35 | 12 | 12–1 | 0.893 | 135 | 6.6 | 47 | Mild grade of intoxication. Moderate diuresis. |
| | | | 1–2 | 0.788 | 121 | 6.5 | | |

*Dog 18–59* (*Table V*).—Died during the night.

*Autopsy.*—All the organs intensely congested with dark purplish blood. Lumen of intestine contains considerable blood-stained watery mucus. Spleen very greatly enlarged and hemorrhagic. Kidneys show considerable postmortem softening. Pancreas partially digested.

*Microscopic Examination.*—Congestion of the viscera. Finer structures blurred by postmortem alterations.

TABLE V.

*Dog 18–59. Large Female Pointer. Injection of Proteose. Effect upon the Ratio* $\frac{\text{Urea in 1 Hr.'s Urine}}{\text{Urea in 100 Cc. of Blood}}$ *and the Excretion of Sodium Chloride.*

*Sodium Chloride Injected.*

| Hour. | Chloride excreted. | Plasma chlorides per 100 cc. | Urea excreted. | Blood urea per 100 cc. | Urea ratio. | Remarks. |
|---|---|---|---|---|---|---|
| | *gm.* | *mg.* | *gm.* | *mg.* | | |
| 9–10 | 0.948 | 678 | 0.127 | 23 | 5.5 | Moderate diuresis after salt injection. |
| 10–11 | 0.600 | 663 | 0.142 | 24 | 5.9 | |
| 11–12 | 0.476 | 630 | 0.118 | 22 | 5.3 | |
| Total..... | 2.024 | | 0.387 | | | |
| Oct. 2, 1918, 10 a.m. 120 cc. of proteose solution intravenously. | | | | | | |
| 11.50–12.50 | 0.166 | | 0.106 | 38 | 2.8 | |
| 12.50– 1.50 | 0.110 | 672 | 0.068 | 53 | 1.3 | Intoxication intense. 12.30 p.m. 150 cc. of 10% sugar solution. |
| 1.50– 2.50 | 0.090 | | 0.032 | 64 | 0.5 | Oliguria continued. |
| 5–6 | —* | 631 | 0.020 | 87 | 0.2 | Dye elimination from 6–8.30 p.m. mere trace. Dog died late during the night. |
| Total..... | 0.366 | | 0.226 | | | |

* Chlorides lost.

An experiment is tabulated in Table V showing the effect of proteose intoxication upon the excretion of injected sodium chloride. After a preliminary fast of 3 days, Dog 18–59, a female adult, weighing 45 pounds, was given 5 gm. of sodium chloride dissolved in 150 cc. of distilled water intravenously. The hourly excretion of chlorides was then determined for 3 successive hours. The plasma chlorides and the urea ratio were also determined. The next day the animal received 120 cc. of a dilute toxic proteose solution intravenously and 1 hour and 50 minutes later 5 gm. of sodium chloride in 250 cc. of distilled water also intravenously, following which the chloride excretion, the plasma chlorides, and the urea ratio were determined.

Reference to the table shows that the total excretion of chlorides following the injection of the proteose was markedly reduced. The plasma chlorides show only an insignificant rise in the 2nd hour above the level in the corresponding hour of the previous day. The plasma chlorides were not determined for each hour as in the control period because the large amounts of fluid injected with the view of helping the animal to survive the intoxication doubtless diluted the plasma to such an extent that figures on the chloride content would be of little value as far as their throwing light upon the question of salt retention is concerned.

The urea ratio was determined as a check on the grade of intoxication and the degree of kidney functional impairment as measured by the change in this ratio. It will be seen that this was lowered to between one-third and one-fourth of its normal value, which fact speaks for a very intense intoxication. However, the decrease in total chloride excretion over the period is much greater than is the decrease in the urea ratio.

*Dog 19–29 (Table VI).—*

*Autopsy.*—Heart and lungs normal. Liver and spleen extremely engorged. Subcapsular hemorrhages in spleen. Gall bladder markedly congested; wall appears like jelly—very edematous and thick. Pancreas very hyperemic. Kidneys slightly engorged, but otherwise negative. Intestinal tract less engorged than usual.

*Microscopic Examination.*—Kidneys normal. Moderate congestion of other organs.

In Table VI are presented two experiments similar to that tabulated in Table V.

Dog 19–29, a small female adult fox-terrier, weighing 13 pounds, after a preliminary fast of 3 days, received 5 gm. of sodium chloride in 200 cc. of distilled water intravenously Oct. 12. The chloride excretion was then determined for the next 24 hour period subdivided into the following periods: first 2 hours; second 2 hours; next 4 hours; and finally the remainder of the 24 hours. The blood urea was determined for the first two subperiods merely to find the normal level for comparison with the values to be obtained in the later experiment. The plasma chlorides were determined at the end of the 1st hour after salt injection and again the next morning near the end of the 24 hour period.

The animal was fed a mixed diet on Oct. 13 and 14, after which the food was withdrawn for a second preliminary fast of 3 days. On Oct. 18, at 10.10 a.m. 26 cc. of a toxic proteose preparation were injected intravenously and at 11.10 a.m., 5 gm. of sodium chloride in 250 cc. of distilled water were given intra-

venously. The chloride excretion, plasma chlorides, and the blood urea were then followed over a 24 hour period subdivided as in the above control period.

The intoxication was very intense, the animal dying about 28 hours after the injection of the poison. The total excretion of chlorides was much reduced. The blood urea mounted to 93 mg. per 100 cc. of blood, even after some dilution by injected fluid. The plasma chloride is slightly higher at the end of the 1st hour after the salt injection and considerably higher near the end of the period than at corresponding times during the preliminary control period.

TABLE VI.

*Injection of Proteose. Chloride Excretion. Sodium Chloride Injected.*

| | Hour. | Chloride excreted. | Plasma chlorides per 100 cc. | Blood urea per 100 cc. | Remarks. |
|---|---|---|---|---|---|
| *1918* | | *gm.* | *mg.* | *mg.* | |
| Dog 19–29, weight 13 lbs. | | | | | |
| Oct. 12 | 9.15–11.15 | 1.806 | 684 | 24 | Moderate diuresis. |
| | 11.15– 1.15 | 1.029 | 644 | 23 | |
| | 1.15– 5.15 | 0.670 | | | |
| | 5.15 p.m.–9.15 a.m. | 1.848 | 611 | | Next day. |
| | Total 24 hrs..... | 5.353 | | | |
| | Oct. 18, 10.10 a.m. | Proteose injected intravenously. | | | |
| " 18 | 11.10– 1.10 | 0.341 | 689 | 36 | Moderate grade of intoxication at first. |
| | 1.10– 3.10 | 0.227 | | 53 | Slight diuresis during 1st few hrs. Oliguria during latter portion of period. |
| | 3.10– 7.10 | 0.208 | | 73 | Intoxication intense later. 200 cc. of 6% acacia and 10% glucose intravenously. |
| | 7.10 p.m.–11.10 a.m. | 0.258 | 661 | 93 | Next day. Rectal temperature subnormal (34°C.) a few hrs. before death. Oct. 19, 1.45 p.m. Killed in terminal stage of intoxication. |
| | Total 24 hrs..... | 1.034 | | | |

TABLE VI—*Concluded.*

| | Hour. | Chloride excreted. | Plasma chlorides per 100 cc. | Blood urea per 100 cc. | Remarks. |
|---|---|---|---|---|---|
| *1918* | | *gm.* | *mg.* | *mg.* | |
| Dog 19–42, weight 28 lbs. | | | | | |
| Oct. 6 | 10.13–11.13 | 0.822 | 670 | | |
| | 11.13– 1.13 | 0.842 | 661 | | Considerable diuresis. |
| | 1.13 p.m.–10.13 a.m. | 3.328 | 561 | | Next day. |
| | Total 24 hrs..... | 4.990 | | | |
| | Oct. 11, 10 a.m. 27 cc. of proteose injected intravenously. 12 m. 5 gm. of NaCl + 10 gm. of urea in 250 cc. of distilled water. | | | | |
| " 11 | 12–1 | 0.743 | 670 | | Mild grade of intoxication. Reaction somewhat delayed. |
| | 1–3 | 0.606 | 663 | | Mild diuresis. |
| | 3 p.m.–12 m. | 1.750 | 611 | | Next day. |
| | Total 24 hrs..... | 3.099 | | | |

Dog 19–42 (Table VI) was given intravenously 5 gm. of sodium chloride dissolved in 250 cc. of distilled water containing 10 gm. of urea at 10.13 a.m., Oct. 6, after a preliminary fast of 3 days. The chloride excretion and the plasma chlorides were then determined for the next 24 hours divided into three subperiods; *viz.*, for the 1st hour after the salt injection; for the next 2 hours; and finally, for the remainder of the 24 hour period. Blood samples for the determination of the plasma chlorides were taken in the middle of each of the first two periods and near the end of the third.

The animal was then fed a mixed diet until Oct. 8, after which the food was withdrawn.

At 10 a.m., Oct. 11, 27 cc. of toxic proteose solution were injected intravenously.

At 12 m., 5 gm. of sodium chloride plus 10 gm. of urea dissolved in 250 cc. of distilled water were given intravenously exactly as in the control period. The chloride excretion and the plasma chlorides were then determined over a 24 hour period subdivided as for the control period.

The urea was injected merely as an added diuretic to insure a sufficient flow of urine.

Even in this mild intoxication there is seen to be a moderate retention of chlorides, more striking for the third subperiod than for the first two. The plasma chlorides for the second subperiod are slightly higher and the next morning considerably higher than at the corresponding times during the control period. Yet, the difference is small and may not be of great significance in comparison with the marked decrease in chloride excretion.

Table VII groups together the experiments on the effect of proteoses from miscellaneous sources upon the blood urea and the elimination of phenolsulfonephthalein. It will be seen from the figures presented that neither the blood urea nor the percentage of dye excreted is much disturbed by comparatively large doses of most of the products used. The various digests were tested at different stages, but, since the results were uniform from time to time, a few representative figures only are tabulated here. These are a unit as far as they go in showing no depression of the renal function.

Most of the products examined possess but slight toxicity as compared with the proteose preparation from the obstruction material. One only produced symptoms of intoxication, when injected intravenously in the dog, comparable with the latter material. This was obtained from a pepsin digest of muscle tissue which contained a rather luxuriant growth of bacteria, including some proteolytic anaerobes, which were able to grow under the thick layer of toluene intended to prevent bacterial growth. Besides a rich content of alcohol-soluble substances which resist heat at boiling temperature and which produce an anaphylactic-like reaction, this particular digestion mixture also contained an alcohol-insoluble substance or group of substances which gave rise to symptoms closely resembling those produced by an injection of the toxic proteose from the contents of an obstructed small intestine or a closed intestinal loop.

The small amount of this alcohol-precipitable material injected for the renal function study caused but a slight decrease in the percentage of dye eliminated, which itself may have been due in large part to extrarenal factors since none of the precautions taken in the majority of the experiments above (Tables I and II) against these was exercised in the case of the experiments presented in Table VII.

Too little is known as yet regarding the nature and the production of this toxic preparation to discuss it further here. It is being inves-

TABLE VII.

*Injection of Proteose Preparations from Various Sources.*

| Source of proteose. | Dog No. and weight. | Quantity of solution injected. | Clinical reaction. | | | Blood urea per 100 cc. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|---|---|---|---|
| | | | Pulse. | Body temperature. | Miscellaneous. | | | |
| | | *cc.* | | °C. | | *mg.* | *per cent* | |
| Commercial Witte's "Peptone." | 19–59<br>49 lbs. | 75 | Greatly depressed. | 38.2<br>39.2 | Dog slightly more dull. | 38 | 64 | 3.2 gm. dry weight. Dog soon recovered. |
| | 19–50<br>27 lbs. | 90 | Greatly depressed. | 38.2<br>38.7 | Dog slightly more dull. | 44 | 67 | 4 gm. dry weight. Dog soon recovered. |
| Pepsin digest of liver tissue (Martin's broth). | 19–42<br>26 lbs. | 200 | Slight transient depression. | 38.1 | Bright and active. | 29 | 71 | Considerable proteose content. |
| Pepsin digest of sterile muscle tissue. | 19–51<br>28 lbs. | 55 | Slight transient depression. | 38.6 | Bright and active. | 33 | 65 | Rich in proteoses. |
| Pepsin digest found to contain proteolytic bacteria. | 19–30<br>22 lbs. | 30 | Moderate depression lasting several hrs. | 38.0<br>40.1 | Very dull. Vomiting. Diarrhea. | 38 | 58 | Proteose precipitated with alcohol. |
| Sterile heart muscle digested by *B. histolyticus*. | 19–51<br>27 lbs. | 40–70 | Moderate transient depression. | 38.0<br>39.4 | Slightly dull. | 30 | 68 | Toxicity slight. Proteose content high. |
| Sterile heart muscle digested by *B. sporogenes*. | 19–50<br>26 lbs. | 40–70 | Moderate transient depression. | 38.0<br>39.8 | Slightly dull. | 32 | 69 | Toxicity slight. Proteose content high. |
| Sterile heart muscle digested by *B. bifermentans*. | 19–51<br>27 lbs. | 30–62 | Moderate transient depression. | 38.4<br>39.4 | Quiet. Salivated. | 34 | 72 | Toxicity slight. Proteose content high. |

tigated from various angles at present, including a further study of its chemical properties and the bacterial flora concerned in its production.

Table VIII shows the effect of a comparatively large dose of Witte's peptone solution on the urea ratio and the elimination of phenolsulfonephthalein. In order that the effect of the proteoses from this source might be compared with that of the toxic proteose obtained from the obstruction material, the determinations were made 2 hours after the

TABLE VIII.

*Dog 19–59. Injection of Witte's Peptone. Effect upon the Ratio* $\frac{\text{Urea in 1 Hr.'s Urine}}{\text{Urea in 100 Cc. of Blood}}$ *and the Elimination of Phenolsulfonephthalein.*

| Date. | Hour. | Urea excreted. | Blood urea per 100 cc. | Urea ratio. | Phthalein elimination in 2 hrs. | Remarks. |
|---|---|---|---|---|---|---|
| *1918* | | *gm.* | *mg.* | | *per cent* | |
| July 9 | 10–11 | 0.167 | 33 | 5.0 | | No food on previous day. |
| | 11–12 | 0.172 | 34 | 5.0 | 72 | |
| July 10 | 10–11 | 0.124 | 24 | 5.1 | | No food for 2 days. |
| | 11.30 a.m. 4 gm. of Witte's peptone dissolved in 80 cc. of water given intravenously. | | | | | |
| | 1.30–2.30 | 0.240 | 38 | 6.3 | | Blood pressure only slightly below normal. Animal bright and active. |
| | 2.30–3.30 | 0.232 | 36 | 6.4 | 70 | |

peptone solution was injected, when the animal, therefore, had almost completely recovered from the effects of the injection. There is seen to be a slight increase in the blood urea and in the quantity of urea excreted per hour. In contrast to the observations made on the relation of the urea excreted per hour to the blood urea in the typical proteose intoxication, there is a slight rise in the ratio, which indicates a normal response on the part of the kidneys. The phthalein elimination is likewise normal.

## DISCUSSION.

The outstanding fact presented in the foregoing tables is that the excretory function of the kidneys is decidedly impaired by the intravenous injection of the toxic material obtained from the contents of the obstructed small intestine.

Another interesting feature of these experiments is that the effect of the toxic substance on the excretory mechanism was found to be only temporary, in most instances disappearing soon after the animal had recovered completely from the intoxication. The suggestion made in the previous report that the degree of impairment of the renal function corresponds fairly closely with the grade of intoxication is further substantiated by these observations.

The demonstration of the latter points explains the observation (8) that the apex of the curve of nitrogen excretion occurs usually on the 2nd day following an injection of toxic proteose instead of on the 1st day when the blood non-protein nitrogen is at its highest level.

The experiments tabulated here likewise explain the enormous increase in the non-protein nitrogen of the blood sometimes observed after proteose injections as being due in part to the retention of this group of substances as well as to an increase in the rate of protein breakdown.

That delay in the absorption of the test dye during the period of intoxication plays no prominent part in the observed failure of the kidneys to eliminate this substance at the normal rate is clearly shown by the experiments presented in Table II in which the phthalein was injected directly into the blood stream. Since comparatively large amounts of fluid were administered during the period of observation following the proteose injections, it seems to us improbable that the factors of lowered blood pressure and concentration of the blood commonly observed in this condition can account for any large part of the temporary defect in the eliminative function.

Most of the other proteose preparations employed in this study fail to cause any appreciable impairment of the renal function as far as they were examined, although they give the same chemical tests given by the proteose material procured from the obstructed intestine. The initial depression of the blood pressure produced by

these miscellaneous proteoses was in every case greater than that caused by injection of the obstruction material. Therefore, this factor can also be dismissed as having no important place in the explanation of the observed facts.

It is evident that all proteoses do not depress the renal function, although some which were tested by Chittenden, Mendel, and Henderson (12) were found to cause anuria without materially affecting the blood pressure and may well have temporarily disturbed the renal function.

We believe that it is more than a coincidence that this proteose mixture obtainable from the obstructed small intestine, but not from the normal intestine, can effect so pronounced a depression of the renal function. Furthermore, the acute intoxication of intestinal obstruction is accompanied by a similar depression of renal function which is likewise transient and capable of swift return to normal if the intoxication is relieved. This is strong evidence that substances resembling exactly those found in the obstructed intestine are wholly responsible for the general intoxication of intestinal obstruction.

There appears to be produced by these toxic substances a primary injury of the secreting cells of the kidney itself which is responsible for the greater part of the impairment as was pointed out in the preceding communication (1), although this injury is not demonstrable by the ordinary histological technique. As far as we are aware this is one of the first instances observed in which a marked kidney injury or impaired function has been demonstrated, followed very quickly by repair and a return to normal, associated with no trace of permanent injury. It seems probable that this type of injury might prove to be more serious if superposed upon a kidney already diseased (chronic nephritis).

## SUMMARY AND CONCLUSIONS.

The injection of the toxic proteose obtained from the contents of the obstructed small intestine causes a definite impairment of the eliminative function of the kidneys as shown by a decreased capacity to excrete urea, sodium chloride, and phenolsulfonephthalein.

This involvement of the renal function is similar to that shown by the preceding report (1) to accompany the intoxication of intestinal obstruction.

The observed depression of function is readily demonstrable even when large amounts of fluid and urea, dye, or salt are injected directly into the blood stream.

There is in all probability a temporary injury of the kidney cells, since the most important extrarenal factors have been largely eliminated in the above experiments.

There is no appreciable impairment of the renal function following the injection of a number of other proteose preparations from a variety of sources.

This study affords new evidence in favor of the view that the function of an organ can be profoundly disturbed for a time without any demonstrable anatomical lesions.

The repair of this type of injury promptly follows the disappearance of the intoxication and is functionally and anatomically perfect.

In conclusion the writers take pleasure in the acknowledgment of aid and cooperation on the part of Dr. H. M. Evans of the Department of Anatomy. Work on vital staining of the kidney and body tissues in conditions of proteose intoxication was taken up by Mr. McQuarrie, working under the supervision of Dr. Evans.

## BIBLIOGRAPHY.

1. McQuarrie, I., and Whipple, G. H., *J. Exp. Med.*, 1919, xxix, 397.
2. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *J. Exp. Med.*, 1913, xvii, 286.
3. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *J. Exp. Med.*, 1913, xvii, 307.
4. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *J. Exp. Med.*, 1914, xix, 144.
5. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *J. Exp. Med.*, 1914, xix, 166.
6. Whipple, G. H., Rodenbaugh, F. H., and Kilgore, A. R., *J. Exp. Med.*, 1916, xxiii, 123.
7. Cooke, J. V., Rodenbaugh, F. H., and Whipple, G. H., *J. Exp. Med.*, 1916, xxiii, 717.

8. Whipple, G. H., and Cooke, J. V., *J. Exp. Med.*, 1917, xxv, 461.
9. Rowntree, L. G., and Geraghty, J. T., *J. Pharmacol. and Exp. Therap.*, 1909–10, i, 579.
10. Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1912, ix, 284.
11. Whipple, G. H., and Van Slyke, D. D., *J. Exp. Med.*, 1918, xxviii, 213.
12. Chittenden, R. H., Mendel, L. B., and Henderson, Y., *Am. J. Physiol.*, 1898–99, ii, 142.

# EFFECT OF BILE ON THE CLOTTING TIME OF BLOOD.

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Although it is known that jaundice tends to cause delayed clotting of blood, the cause of the delay is not known. Minot and his associates,[1] using Howell's method of recalcifying oxalated plasma, found that the coagulation time (prothrombin time of Howell) was increased in a series of jaundice cases, but did not suggest an explanation of the mechanism of the delay in coagulation. It seemed of interest, therefore, to determine whether or not bile or bile salts, which are, of course, present in the blood in jaundice, are in themselves capable of causing the increase in the coagulation time.

## *Effect of Bile on Clotting of Plasma.*

*Series 1.*—Cats, under ether anesthesia, were bled from a large artery, through a paraffined cannula into paraffined 50 cc. centrifuge tubes containing 7.5 cc. of 1 per cent sodium oxalate in 0.9 per cent sodium chloride solution. The tubes were then centrifuged and the plasma was carefully pipetted off. If the plasma showed the least trace of hemolysis it was rejected. A series of flat bottom tubes, 22 mm. in diameter, was then set up, each containing 2 cc. of plasma and 0.5 cc. of an ox bile solution of varying concentration. To each of the tubes an amount of calcium chloride was added which had been previously found to produce a firm clot in the minimum time with the same plasma. The time necessary for the formation of a firm clot in each tube was recorded. Precipitation of fibrin was considered complete when a clot of such consistency was formed that the tubes could be inverted without loss of liquid. As shown in Table I, clotting time was delayed in proportion to the amount of bile present

[1] Minot, G. R., Denny, G. P., and Davis, D., *Arch. Int. Med.*, 1916, xvii, 101.

TABLE I.

2 cc. of oxalated plasma plus 0.5 cc. of bile solution, in dilution to give the final concentration indicated, were recalcified with the optimum quantity of calcium chloride solution.

| Bile. | Clotting time. | Bile. | Clotting time. |
|---|---|---|---|
| *per cent* | *min.* | *per cent* | *min.* |
| 9.0 | No clot. | 4.0 | 5 |
| 8.0 | " " | 3.3 | 4 |
| 7.7 | " " | 2.5 | 4 |
| 7.4 | 52 | 2.0 | 4 |
| 6.9 | 32 | 1.0 | 3 |
| 6.6 | 27 | 0.0 | 3 |
| 5.0 | 14 | | |

in the plasma. The results were uniform with all the animals in this series.

Similar results were obtained when whole oxalated blood was used to which varying quantities of bile had been added.

*Effect of Sodium Glycocholate on Clotting of Plasma.*

*Series 2.*—In these experiments a solution of sodium glycocholate was substituted for bile. The experiments were otherwise identical with those of Series 1. Table II, the record of a typical experiment of this series, shows that results are similar to those of the first set.

We may conclude that bile and bile salts, in sufficient quantity, retard the coagulation of blood. Most modern workers conceive of the process of coagulation as taking place in two steps: first, the formation of thrombin; and second, the conversion of fibrinogen into

TABLE II.

0.5 cc. of oxalated plasma, containing the indicated concentration of glycocholate, was recalcified with the optimum quantity of calcium chloride solution.

| Sodium glycocholate. | Clotting time. | Sodium glycocholate. | Clotting time. |
|---|---|---|---|
| *per cent* | *min.* | *per cent* | *min.* |
| 0.5 | 13 | 0.2 | 5 |
| 0.4 | 9 | 0.1 | 4 |
| 0.3 | 5 | 0.0 | 5 |

fibrin by the action of thrombin. To determine whether the bile salts prevented the formation of thrombin or merely interfered with the change of fibrinogen to fibrin even though an adequate supply of thrombin was present, the effect of bile and bile salts on the clotting time of a solution of fibrinogen in the presence of sufficient quantities of preformed thrombin was tested in Series 3 and 4. Solutions of fibrin and thrombin were prepared according to the method of Mellanby.[2] Not only is this method simple and rapid, but the other clotting elements which are present in small amounts do not interfere.

*Preparation of Fibrinogen.*—Fowl plasma was used because it may be kept for weeks without spontaneous clotting. Fibrinogen was precipitated out by diluting the plasma with ten volumes of distilled water and neutralizing with a few drops of 1 per cent acetic acid. The precipitate was collected and dissolved in 0.5 per cent sodium chloride solution, about 40 cc. being a convenient amount when 50 cc. of plasma were originally used. Since this solution clotted upon addition of both thrombokinase and calcium, but not upon addition of either alone, it was concluded that it contained fibrinogen and prothrombin.

*Preparation of Thrombin.*—10 cc. of the above solution were coagulated by the addition of a trace of thrombokinase (extract of chick embryo) and calcium chloride to a concentration of 0.05 per cent. After removal of fibrin, the clear, residual fluid contains a quantity of thrombin proportional to the strength of the original fibrinogen-prothrombin solution, and a trace of thrombokinase. It is not essential to have thrombin free of other elements, provided that sufficient thrombin is available in solution and that there are no substances present which interfere with its action.

### *Effect of Bile and Bile Salts on Clotting of Fibrinogen in the Presence of Thrombin.*

*Series 3.*—In these experiments 0.5 cc. of a solution of fibrinogen was used, to which bile had been added to the desired concentration. An excess of thrombin was then added and the clotting time noted. The result of one such experiment is recorded in Table III.

[2] Mellanby, J., *J. Physiol.*, 1917, li, 396.

*Series 4.*—Experiments in this series were carried out in the same way, except that sodium glycocholate was substituted for ox bile (Table IV).

TABLE III.

0.5 cc. of fibrinogen solution plus enough bile to make the indicated final concentration.

| Bile. | Clotting time. | Bile. | Clotting time. |
|---|---|---|---|
| *per cent* | *min.* | *per cent* | *min.* |
| 13 | No clot. | 6 | 15 |
| 11 | Loose clot. | 5 | 7 |
| 9 | " " | 4 | 2 |
| 7 | 21 | 3 | 2 |

TABLE IV.

0.5 cc. of fibrinogen solution plus sodium glycocholate.

| Sodium glycocholate. | Clotting time. | Sodium glycocholate. | Clotting time. |
|---|---|---|---|
| *per cent* | *min.* | *per cent* | *min.* |
| 1.2 | No clot. | 0.6 | 7 |
| 1.0 | Loose clot. | 0.5 | 4 |
| 0.8 | 14 | 0.4 | 3 |
| 0.7 | 10 | 0.0 | 4 |

*Effect of Sodium Oleate on Clotting of Fibrinogen in the Presence of Thrombin.*—A few experiments were also performed with sodium oleate instead of bile salts. Sodium oleate, which is the salt of an unsaturated fatty acid, is somewhat similar to sodium glycocholate in physical properties. Small amounts did not interfere with the clotting time; very large amounts inhibited clotting entirely; and moderate amounts caused delayed clotting. In no experiment, however, was there the same gradual increase in clotting time with increase of the salt which was observed in experiments with bile or sodium glycocholate. The superficial resemblances of the results, however, suggested that the mechanism may be the same as in the case of bile salts.

## DISCUSSION.

It is evident from the above experiments that within certain limits clotting time depends on the percentage of bile present in solution and that the reaction is the same in experiments with pure solutions

of the substances concerned in coagulation, as in whole plasma. It likewise seems justifiable to conclude that bile and bile salts do not interfere with the formation of thrombin, since the prolongation of clotting time is just as great when preformed thrombin is added in ample quantity to fibrinogen solution, as when thrombin must be formed from its precursors in the presence of bile. It cannot be a question of destruction of the thrombin, as Morawitz and Bierich[3] showed that a quantity of freshly drawn blood which had been mixed with enough bile to inhibit clotting, could be caused to coagulate by merely diluting the mixture with isotonic salt solution. Consequently we must assume that it is the conversion of fibrinogen to fibrin that is interfered with rather than the formation of thrombin.

In our experiments it was found that there was a retardation of clotting, great enough to be detected by clinical methods, with amounts of bile greater than 5 per cent. We were unable to find reports in the literature stating the exact amounts of bile salts present in the blood in jaundice. Gilbert[4] states that in cases of obstructive jaundice bile pigment is present in the blood in quantities of from 0.7 to 1 gm. per liter. Bile itself contains about 1 gm. of pigment per liter. The relation of bile pigments and bile salts in the blood in jaundice has not been determined, but it would seem possible for the salt to be present in sufficient concentration to prevent clotting.

## CONCLUSIONS.

1. Within certain limits the clotting time of blood, of blood plasma, and of solutions of fibrin to which bile salts have been added, is proportional to the quantity of bile present.

2. The bile interferes with the conversion of fibrinogen into fibrin and not with the formation of thrombin.

[3] Morawitz, P., and Bierich, R., *Arch. exp. Path. u. Pharmakol.*, 1907, lvi, 115.

[4] Gilbert, quoted by Wells, H. G., Chemical Pathology, Philadelphia and London, 3rd edition, 1918, 486.

# A CHARACTERISTIC LOCALIZATION OF BACILLUS ABORTUS IN THE BOVINE FETAL MEMBRANES.

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Plates 20 to 22.

(Received for publication, February 21, 1919.)

While making a study of the diseased membranes in cases of infectious abortion of cattle, the writer came upon a peculiar and characteristic habitat of *Bacillus abortus* Bang, in the epithelial covering of the chorion.

This layer of cells which faces the epithelial covering of the uterine mucosa and is in intimate contact with it covers the intercotyledonous areas of the chorion and is continuous with the epithelium of the villosities of the cotyledons which dip into the depressions of the maternal caruncles. The general character of the cells may be seen in Fig. 4. The cells vary somewhat in height. The vesicular nucleus is round or oval in outline and the chromatin appears as minute 1 $\mu$ spheres against the nuclear membrane. The free border of the cytoplasm appears frequently in the form of blunt finger-like or conical projections, giving the surface a fimbriated appearance. In the specific infectious disease of the fetal membranes these cells, either individually or in series, are densely filled with minute bacilli. The invasion is recognizable under a low power in that the cytoplasm of the affected cell assumes a blue color when the section is stained in eosin-methylene blue (Fig. 1). High powers resolve this tint into fine, short, rod-like bodies (Figs. 2 to 6). The bacilli do not lie on the cell or in the ectoplasm but fill the cell body entirely. When the microscope is raised or lowered the cytoplasm appears filled in all optical sections.

The host cells are more or less altered as to size and condition of nucleus. Some cells are still normal in size and the nucleus is recog-

nizable as a vesicle with contained chromatin (Fig. 4). In others the nucleus is compact, pyknotic. The cell body becomes enlarged and it may measure up to 40 $\mu$ in diameter (Fig. 6). Vacuolation of the cytoplasm is not uncommon (Fig. 3). Fetal membranes at the end of the normal period of gestation which are macroscopically without pathological changes have been found uniformly free from such cell contents. The same is true of fetal membranes from cases of abortion associated with spirilla from which *Bacillus abortus* is not obtainable, either in cultures or through guinea pig inoculation.[1] In all cases in which the invasion of the epithelial cells was detected this feature was associated with positive cultures of *Bacillus abortus*, or the characteristic guinea pig disease after inoculation, or with both tests positive. Simple cover-slip preparations from the exudate or the necrotic villi were frequently sufficient to demonstrate the presence of infected epithelia. Besides the chorionic epithelium, the epithelial cells at the margin of the cotyledons and those of the outermost villi of the latter have been found invaded (Figs. 2 and 5). The bulk of the villi though undergoing profound changes are as a rule free from nests of bacteria.

That the cell localization described above is a regular occurrence wherever *B. abortus* is active may be gathered from the scanty literature by an interpretation of certain statements made, although none of the authors to be cited made an attempt to determine the precise source of the clumps of bacilli. Thus Bang[2] in 1897 describes the bacteria in films from the fetal membranes as either free or in dense clumps which appeared to have been formed inside cells. The following statement occurs in the British report:[3] "In many places the bacilli are collected into dense groups or colonies. Some of these groups look as if they were bounded by a cell membrane and give the impression of being contained inside tissue cells."[4] It is also stated[5] that: "It is an easy matter to identify the characteristic clumps of abortion bacilli in microscopic preparations made from the uterine exudate discharged immediately before or after abortion." In the Appendix[6] we read as

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[1] Smith, T., *J. Exp. Med.*, 1918, xxviii, 701.

[2] Bang, B., *Z. Thiermed.*, 1897, i, 241.

[3] Great Britain Board of Agriculture and Fisheries, Report of the departmental committee on epizootic abortion, London, 1909, pt. 1.

[4] British report,[3] p. 6.

[5] British report,[3] p. 15.

[6] British report,[3] Appendix, p. 27.

follows: ". . . . so far as the membranes and swabs are concerned a positive diagnosis was based on the presence of the specific microbe in the typical clump form because these materials were always very impure and it is not possible to identify scattered abortion bacilli in a mixture of bacteria." Zwick and Zeller[7] refer to bacilli in large numbers in uterus exudate and state that cells may be filled with them. Fig. 3 illustrates very well what these writers probably refer to in most instances. In some cases, however, they may have seen leucocytes which at times are quite numerous and densely filled with bacilli. The latter are probably set free from the detached and disintegrated epithelial cells and taken in by leucocytes.

The mode of invasion of the epithelium by bacteria manifesting no true motility but only Brownian motion may be tentatively explained by assuming that the bacteria entering the uterochorionic space by way of the blood vessels in the uterine wall adhere to the ectoplasm and are rubbed into the substance of the cell by the pressure exerted by the uterine wall on the chorion. Once in the cytoplasm the bacteria find it a favorable medium for multiplication and a protection against phagocytosis. An alternative explanation would be to ascribe ameboid activities to the chorionic epithelium, for which theory at present no adequate proof exists.

It may be maintained that the cell parasitism is not an active disease process but rather secondary to it and operating only on cells partly or wholly devitalized. This interpretation has been kept in mind in the study of the various cases. When the disease is so far advanced that expulsion of the immature fetus occurs, the pathological material may fail to furnish an unequivocal answer to the above question. In several cases, however, in which a living calf at full term had fetal membranes in the early stages of infection, and in one slaughtered cow, the appearance of the invaded cells and the irregular distribution of the infection make it probable that they had been invaded while still in a normal condition (Figs. 2, 4, and 5).

The other types of epithelia, such as those of the uterine mucosa and of the amnion, have not been found invaded. The uterine epithelium resembles in its columnar appearance that of the chorion, but the cytoplasm forms a smooth, level, uninterrupted surface. The amniotic epithelium is of the flat, squamous type.

[7] Zwick and Zeller, *Arb. k. Gsndhtsamte.*, 1913, xliii, 1.

SUMMARY.

The significance of this invasion of the chorionic epithelium from the standpoint of pathogenesis cannot be properly evaluated until a more complete history of the successive localizations of *Bacillus abortus* has been obtained. It is safe to assume that this particular cell parasitism is but one of a series of localizations and centers of multiplication in the fetal membranes although evidence points to it as perhaps the earliest stage in which the organism gains by rapid, unchecked multiplication a considerable advantage over the host. The local destruction of an epithelial covering by an infectious agent when other miscellaneous infectious agents are absent may or may not be of much importance, for it would depend on the regenerative activity of the epithelium, the tendency to the gathering of injurious transudates, and the toxic substances associated with the bacilli.

It is probable that localizations also occur in the walls of the blood vessels of the chorion. Thus far only one case of this kind has been observed. The fusiform connective tissue cells of the adventitious coat of a blood vessel 0.8 mm. in diameter were completely replaced by clumps of minute bacilli. Since there is usually a slight perivascular cell infiltration in the diseased placenta this localization may be largely responsible for the circulatory disturbances which lead to death of the fetus. The case referred to may be but a greatly exaggerated illustration of the action of *Bacillus abortus* in the walls of the blood vessels where they are too few in number at any one time to be identified. It is known,[8,9] that in the guinea pig disease with pronounced lesions *Bacillus abortus* is demonstrated only with great difficulty because of its scarcity.

The more or less specific localization and multiplication of bacteria within cells not having phagocytic functions have thus far been demonstrated in leprosy, syphilis, and in a disease of mice recently described by Tyzzer[10] who found an active invasion of both liver cells and intestinal epithelium by a bacillus. In cells to which phagocytic powers have been ascribed the specific localization of certain bac-

[8] Smith, T., and Fabyan, M., *Centr. Bakteriol., Ite Abt., Orig.*, 1912, lxi, 549.

[9] Fabyan, M., *J. Med. Research*, 1912, xxvi, 441.

[10] Tyzzer, E. E., *J. Med. Research*, 1917–18, xxxvii, 307.

teria is well known. Thus tubercle bacilli occur within the endothelial cells of the tubercle. Leprosy bacilli have been found within a variety of cell groups. Mouse septicemia bacilli occur regularly within certain phagocytic cells of the blood.[11] Recently bacteria have been found attached to the cilia of the respiratory tract in pertussis by Mallory and Hornor,[12] in a form of guinea pig pneumonia by the writer.[13] Actual occupation of epithelial cells followed by active multiplication of the invaders and destruction of the cell has, however, been frequently demonstrated for the sporozoa. That it may occur more often among bacteria is highly probable. Rapidity of multiplication and cell destruction or invisibility or both may stand in the way of a satisfactory demonstration.

## EXPLANATION OF PLATES.

### PLATE 20.

FIG. 1. Placenta of Heifer 203, which gave birth to a small but apparently healthy calf. Period of gestation unknown. The epithelium covering the chorion is enlarged, irregular in outline, the cytoplasm stained blue, and the nuclei are pyknotic. The cytoplasm of all is densely packed with bacilli not recognizable at this magnification. *B. abortus* was isolated from the placenta through guinea pigs. 10 days later the calf, having scoured somewhat, was killed and *B. abortus* was isolated from small foci of bronchopneumonia through guinea pigs. × 125.

FIG. 2. Placenta, Cow 146, aborted. A marginal villus of a cotyledon cut transversely and showing two epithelial cells *in situ* densely filled with bacilli. One cell is enlarged, projecting, the other very flat, cut transversely, and showing as a slender, nematode-like body. The nucleus of this latter infected cell is shown. × 1,000.

FIG. 3. Film made from placental exudate, Cow 298, aborted. Alkaline methylene blue. The epithelial cell is enlarged, vacuolated, and filled with bacilli. × 1,000.

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[11] Koch, R., Untersuchungen über die Aetiologie der Wundinfectionskrankheiten, Leipsic, 1878.

[12] Mallory, F. B., and Hornor, A. A., *J. Med. Research*, 1912–13, xxvii, 115.

[13] Smith, T., *J. Med. Research*, 1913–14, xxix, 291.

PLATE 21.

FIG. 4. Placenta, Cow 171. Fetus obtained *in utero* after slaughter. The chorionic epithelium is shown *in situ*. One densely infected cell in the center, others, right and left. The cell nuclei are no longer of normal appearance but the cells themselves are only slightly swollen. × 1,000.

FIG. 5. Placenta, Cow 91, aborted. A short blunt fetal villus from the margin of a cotyledon is shown with all of the covering epithelium filled with bacilli. × 1,000.

PLATE 22.

FIG. 6. Placenta, Cow 127, which gave birth to a full term calf. The epithelial cells of the chorion are slightly lifted away from their normal base. The cells are much larger than normal, the nuclei pyknotic. All are densely packed with bacilli. × 1,000.

Guinea pigs inoculated with placental tissue of all the above cases, ground up and suspended in salt solution, became diseased and yielded pure cultures of *B. abortus*. In several cases cultures were obtained directly in spite of the soiled condition of placenta.

# RESULTS OF PROPHYLACTIC VACCINATION AGAINST PNEUMONIA AT CAMP WHEELER.*

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(Received for publication, February 15, 1919.)

## INTRODUCTION.

The recently published work of Cecil and Austin (1) on the results of prophylactic inoculation against pneumonia at Camp Upton has furnished considerable evidence that vaccination against Type I, II, and III pneumococci is practical and that it affords satisfactory protection against the pneumonia produced by these types. In view of the widespread prevalence of pneumonia in the American Army, especially in the cantonments, it was obvious that immediate effort should be made toward instituting a more extensive trial of pneumococcus vaccine and, if the results justified it, the establishment of pneumonia vaccination on an efficient and permanent basis.

For the sake of convenience, it might be well to refer briefly to the results obtained by Cecil and Austin at Camp Upton.

From a study of the agglutinins and protective power of the serum of 42 persons vaccinated against pneumococcus, Types I, II, and III, it was demonstrated that a definite immune response could be secured to Types I and II by the dose of vaccine employed. Little evidence of response to Type III could be demonstrated by these methods, but this was of less significance in view of the fact that in animals it is relatively difficult to secure antibodies against this strain even though a considerable degree of active immunity may have been produced in the vaccinated animal. The degree of response to the vaccine appeared to be dependent upon the total dose of each type of pneumococcus ad-

*An official report submitted to the Surgeon General by the Commission appointed to investigate pneumococcus vaccination.

ministered. The greater the dose the greater was the protection elicited, and this remained true whether the vaccine was administered in a single large dose, or in a number of successive small doses. 12,519 men were vaccinated at Camp Upton (40 per cent of the camp strength) most of the men receiving three or four inoculations at intervals of 5 to 7 days. The total dosage was 6 to 9 billion of Types I and II and 4½ to 6 billion of Type III. The local and general reactions were usually mild, but there were quite a few small sterile fluctuating infiltrations at the site of the injection which appeared to be an expression of cutaneous hypersusceptibility. The results of the vaccination were highly satisfactory. The men were under observation for 10 weeks following vaccination, and, during that time, no cases of pneumonia of the three fixed types occurred among the men who had received two or more injections of vaccine. In a control of approximately 20,000 men, there were 26 cases of pneumonia of Types I, II, and III during the same period. The incidence of Pneumococcus Type IV pneumonia and streptococcus pneumonia was much less in the vaccinated troops than among the unvaccinated. The final figures showed only 17 cases of pneumonia of all types occurring among the 12,519 men who received vaccine, whereas, among the unvaccinated troops, during the same period, there was a total of 173 cases of pneumonia of all types. For the 10 weeks during which the men were under observation, the annual pneumonia death rate for vaccinated troops was only 0.83 per 1,000; for the unvaccinated troops it was 12.8.

In spite of the successful results obtained at Camp Upton, there were certain objections to pneumococcus vaccine, which interfered somewhat with its extensive application. In the first place, three injections were necessary in order to obtain a satisfactory protection. This, however, placed a great burden on regimental surgeons, and was distasteful to the men themselves as they had already received the triple typhoid injections and the smallpox vaccination. Another objection to pneumococcus vaccination was the occurrence of the small sterile infiltrations which sometimes followed its use. These infiltrations were never serious, but caused some anxiety and discomfort to the patient. It was a fortunate coincidence, therefore, that just at this time Colonel Eugene R. Whitmore (2), of the Medical Corps, U. S. Army, made his first report on the use of vegetable oil as a vehicle for the suspension of bacterial vaccines. Some work had already been done along this line in France by Le Moignic and Pinoy (3) who had tried suspending triple typhoid vaccine in vegetable oils, and had met with promising results. Achard and Foix (4) also reported favorably on the use of olive oil as a medium for

suspending bacteria. Whitmore applied this principle to a wide variety of vaccines, including the pneumococcus. These vaccines differed in no essential respect from the saline vaccine, except that the dried bacteria were suspended in olive oil instead of in salt solution. More recently Colonel Whitmore has substituted cottonseed oil containing 2 per cent lanolin for olive oil, as it appears to be less irritating to the subcutaneous tissue. Whitmore, Fennel, and Petersen (2) found that the triple typhoid vaccine in oil produced even better agglutination response in the serum than the same vaccine in saline solution. The reaction, both local and general, following the injection of the typhoid lipovaccine was so mild that the three original doses of saline vaccine could be combined into one dose (1 cc.) of the lipovaccine, without producing any unpleasant results. This mild reaction was probably due to the fact that the oily suspension was more slowly absorbed than the saline suspension. The reduction of typhoid vaccination to one injection presented such an obvious advantage over the old method that the Surgeon General shortly afterwards directed that the lipovaccine be substituted for the saline vaccine altogether, and it is now being universally employed throughout the Army.

Following the work with typhoid lipovaccine, experiments were undertaken at the Army Medical School with pneumococcus lipovaccine, and here again, it was found that pneumococcus vaccine in oil produced good immune reactions, and that large doses could be administered without ill effect. It was therefore decided to prepare, in addition to the typhoid lipovaccine, a pneumococcus lipovaccine of Types I, II, and III, and to supply it to the Army for vaccination of volunteers. Some preliminary experiments carried out by us at Camp Wheeler had shown that 10 billion pneumococci of each of the three types could be injected without producing severe reactions.

About September 1, 1918, the writers of this report were appointed by the Surgeon General as a special commission to investigate the value of pneumonia vaccine, and were directed to proceed to Camp Wheeler, Georgia, for the purpose of instituting voluntary vaccination against pneumonia among the troops. There were good reasons for the selection of Camp Wheeler for this experiment. During the 6 months from October 5, 1917, to March 29, 1918, there had been 917

TABLE I.

*Comparison of the Cases of Pneumonia from July 1 to October 1 (Summer Epidemic), with Those from October 1 to December 20, 1918 (Influenza Epidemic).*

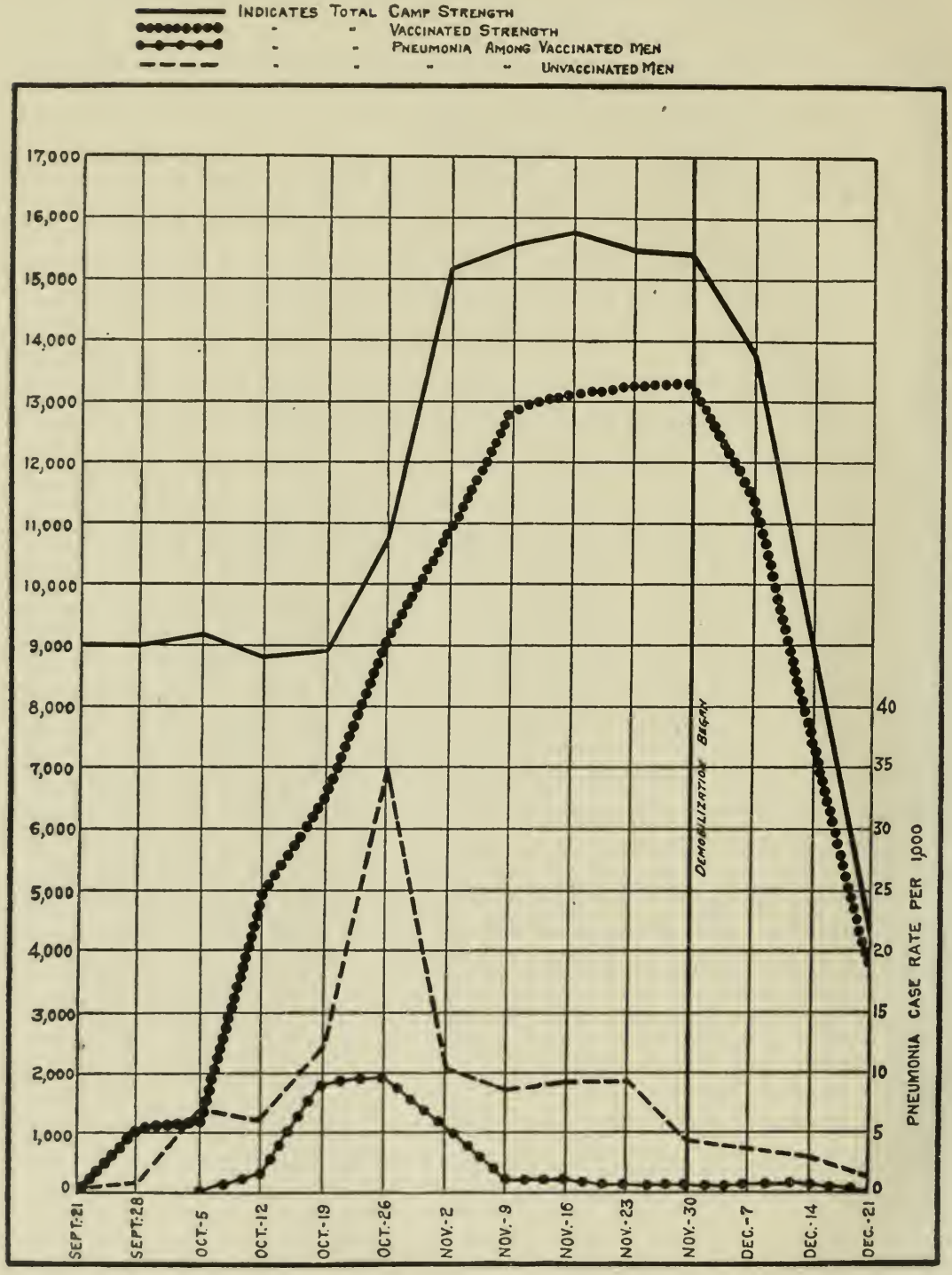
| Types. | July 1 to Oct. 1, 1918. | | | | Oct. 1 to Dec. 20, 1918. | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of cases. | | No. of deaths. | Mortality. | No. of cases. | | No. of deaths. | Mortality. |
| | | | | *per cent* | | | | *per cent* |
| Pneumococcus, I | 41 | | 10 | 24.3 | 13 | | 4 | 30.7 |
| " II | 21 } 73 | (43.2%) | 6 } 18 | 28.6 } 24.6 | 18 } 70 | (11.5%) | 7 } 23 | 38.9 } 32.8 |
| " III | 11 | | 2 | 18.2 | 39 | | 12 | 30.7 |
| " IV | 96 | (56.8%) | 12 | 12.5 | 536 | (88.5%) | 117 | 21.6 |
| Hemolytic streptococcus | 1 | | 0 | 0 | 10 | | 5 | 50.0 |
| Non-hemolytic " | 12 | | 1 | 8.3 | 34 | | 2 | 5.8 |
| Friedländer's bacillus | 0 | | 0 | 0 | 1 | | 1 | 100 |
| Undetermined | 34 | | 3 | 8.9 | 15 | | 2 | 13.3 |
| Total | 216 | | 34 | 15.7 | 666 | | 150 | 22.4 |

cases of pneumonia at Camp Wheeler, a figure which gave that camp the next highest pneumonia rate of all the camps for that period. Furthermore, in August, 1918, a second epidemic of pneumonia broke out at Camp Wheeler among the negro draft and was the immediate cause of our being sent to that camp.

In Table I it will be seen that for the 3 months, July 1 to October 1, 1918, though it was the hot season, there were 216 cases of pneumonia at Camp Wheeler with 34 deaths. This was nearly all primary pneumonia of the lobar type, and practically all of it of pneumococcus origin. Of the pneumococcus cases, 43.2 per cent were due to the fixed types and 56.8 per cent to Type IV. Nearly half of the pneumonia, therefore, was being caused by the types of pneumococcus against which we proposed to vaccinate.

It soon became apparent, however, that conditions at Camp Wheeler were very different from those which had prevailed at Camp Upton the preceding winter. In the first place, many of the men whom we were to vaccinate at Camp Wheeler were raw recruits of rural origin, a considerable part of them negroes. The men vaccinated at Camp Upton had come chiefly from New York City, were well seasoned at the time of vaccination, and were mostly of the white race. In the second place, the situation at Camp Wheeler was complicated by the influenza epidemic, which, of course, had been absent at Camp Upton.

By referring to Text-fig. 1, it will be seen that on September 21, 1918, the date on which vaccination of troops at Camp Wheeler was initiated, the total strength of the camp was about 9,000 men. The population remained practically unchanged until October 16 when a draft of 1,270 negroes arrived. During the following 2 weeks a large draft of white troops arrived, some from New York State and others from Alabama. By November 2, the population of the camp had increased from 9,000 to something over 15,000, and by November 16, the maximum population, nearly 16,000, was reached. On November 30, demobilization began, and during the next 3 weeks the population was reduced from 15,000 to 4,000 men. The troops who constituted the camp in September and the earlier part of October were practically all seasoned men. We succeeded, as shown by the chart, in getting 75 per cent of the old troops vaccinated before the draft arrived.




TEXT-FIG. 1. Pneumonia at Camp Wheeler from September 21 to December 21, 1918.

The new draft began on October 16 and was completed by November 2. The incoming men were examined by a medical officer as they got out of the train. Any men who were sick were sent at once to the Base Hospital. All the others were marched directly to the vaccinating pavilion, and received at once their pneumococcus vaccine. In this way most of the recruits were vaccinated against pneumonia within 24 hours after arriving at camp. All those who objected to taking the vaccine were passed by.

*Technique of Vaccination.*

The men were arranged in single file and instructed to roll up the left sleeve. As the troops filed into the vaccinating building, a record of each man's name, age, race, organization, rank, nativity, and duration in service was taken on one of the so called "Sick and wounded cards" (Form 52). On the lower part of the card was stamped the date and dosage of vaccination. After giving the necessary information, the men passed down a narrow aisle and were vaccinated as follows: An enlisted man swabbed the posterior aspect of the left arm half way between the elbow and shoulder with tincture of iodine. A medical officer then injected 1 cc. of pneumococcus vaccine subcutaneously, using a needle of fairly small caliber. Then, in order to prevent the escape of any of the oily suspension, another enlisted man pressed a piece of absorbent cotton against the point of puncture and instructed the man to hold it there for a minute or two.

In addition to the pneumococcus vaccine 2,226 recruits received an injection of influenza vaccine (1 billion influenza bacilli) in the right arm. The results of the vaccination against influenza will be considered in a separate report. The reaction produced by influenza vaccine was almost always very mild. After the men had received the vaccine, they were marched to their quarters and given complete rest for 24 hours.

By referring again to Text-fig. 1, it will be observed that the recruits were vaccinated almost as rapidly as they came to camp and that by November 9, 13,000 men in all had received pneumococcus vaccine, about 80 per cent of the camp strength. In other words, from October 19 to December 20, a period of 2 months, 75 to 80 per

cent of the entire population of the camp consisted of men who had been vaccinated against pneumonia.

Table II indicates the number of men vaccinated and classified according to whether they were white or negro, recruits or seasoned men. We arbitrarily defined the term "recruit" to mean any man who had been in the service 1 month or less. Table II shows that altogether 3,832 white recruits (80 per cent of the total number) and 1,230 negro recruits (86 per cent of the total number) received the pneumococcus vaccine, making a grand total of 5,062 recruits who were inoculated. Of the seasoned men, 6,687 white troops (75 per

TABLE II.

*Data on Pneumococcus Vaccination.*

| Race. | No. vaccinated. | | | Approximate No. unvaccinated. | | |
|---|---|---|---|---|---|---|
| | Recruits.* | Seasoned men. | Total. | Recruits. | Seasoned men. | Total. |
| White | 3,832 | 6,687 | 10,519 | 919 | 2,156 | 3,075 |
| Negro | 1,230 | 1,711 | 2,941 | 194 | 146 | 340 |
| Total | 5,062 | 8,398 | 13,460 (80% of camp strength). | 1,113 | 2,302 | 3,415 (20% of camp strength). |

* All men in service for 1 month or less were classified as recruits.

cent) and 1,711 negro troops (92 per cent), a total of 8,398, were inoculated against pneumonia. Altogether, 10,519 white men and 2,941 negroes were vaccinated with the pneumococcus lipovaccine, making a grand total of 13,460 men, or 80 per cent of the entire camp strength.

For the purposes of control, it would have been more desirable to have vaccinated only half of the camp; that is, only half of each organization. As this method, however, was not feasible, and in consideration of the serious influenza epidemic, it was decided to vaccinate as large a percentage of the camp as possible with the hope of reducing the pneumonia rate to a minimum.

*Character of Reactions to Pneumococcus Lipovaccine.*

*Local Reaction.*—Pneumococcus lipovaccine produces a mild local reaction, which is distinctly less marked than that caused by triple typhoid lipovaccine, though the former contains more than three times as many bacteria as the latter. That the lipovaccine is much less irritating than the saline vaccine is evidenced by the fact that while the saline preparation often produces a sterile fluctuating infiltration at the site of injection, only five such reactions (Table III) were observed among the 13,000 men vaccinated with lipovaccine at Camp Wheeler. This figure is in strong contrast to the 152 infiltrations encountered in the Camp Upton experiment. This type of local reaction is never serious, but causes some concern and discomfort to the patient, and its frequent occurrence would certainly militate against the general use of pneumococcus vaccine. In our opinion, the elimination of these infiltrations marks one of the most important advances in prophylactic pneumococcus vaccination.

Doses of pneumococcus lipovaccine three times as large as the dose finally decided upon were administered to volunteers at Camp Wheeler without any serious local or general reaction. It is almost incredible that such huge numbers of pathogenic bacteria (90 billion) can be injected beneath the skin with so little untoward effect.

*Constitutional Reaction.*—Like the local reaction, the constitutional reaction to pneumococcus lipovaccine is usually insignificant. In many cases it is entirely absent. Of the 13,460 men who received the vaccine at Camp Wheeler, only 104 (0.7 per cent) were sufficiently affected to be admitted to the Base Hospital (Table III). It is especially interesting to note that while 5,062 recruits and 8,398 seasoned men were inoculated, 101 of the admissions to the hospital were recruits, and only 3 were seasoned men, a fact which would indicate that practically 100 per cent of men in good physical condition can take the vaccine without any particular discomfort.

It is also important to note that 80 of the 104 admissions were among the 2,226 recruits (44 per cent) who received the pneumococcus and influenza vaccine simultaneously; only 24 occurred among the 2,836 recruits (56 per cent) who received pneumococcus vaccine alone.

The negroes seemed more susceptible to the pneumococcus toxin than whites; 65 per cent of the hospital admissions were negroes,

TABLE III.

*Hospital Admissions for Pneumococcus Vaccine Reactions.*

| | |
|---|---|
| Severe local reactions | 5 cases. |

General reactions.

| | Recruits. | Seasoned men. | Total. |
|---|---|---|---|
| White | 34 | 2 | 36 |
| Negro | 67 | 1 | 68 |
| Total | 101 | 3 | 104 |

Day of admission.

| | |
|---|---|
| Day of vaccination | 9 cases. |
| " after " | 48 " |
| 2 days after " | 23 " |
| 3 " " " | 13 " |
| 4 " " " | 5 " |
| 5 " " " | 6 " |
| Total | 104 " |

Symptoms.

| | | |
|---|---|---|
| Headache | 88 cases | (84 per cent). |
| Backache | 37 " | (35 " " ). |
| General muscular aching | 39 " | (38 " " ). |
| Malaise | 22 " | (21 " " ). |
| Chill | 21 " | (20 " " ). |
| Pain in chest | 18 " | (17 " " ). |
| Cough | 13 " | (12 " " ). |
| Sore throat | 11 " | (10 " " ). |
| Nausea | 2 " | |
| Anorexia | 1 " | |
| Nosebleed | 1 " | |

Length of time in hospital.

| | |
|---|---|
| 1 day | 7 cases. |
| 2 days | 30 " |
| 3 " | 22 " |
| 4 " | 13 " |
| 5 " | 9 " |
| More than 5 days | 23 " |
| Total | 104 " |

Fever cases.

| Temperature. °F. | |
|---|---|
| 98– 99 | 12 cases. |
| 99–100 | 14 " |
| 100–101 | 15 " |
| 101–102 | 23 " |
| 102–103 | 17 " |
| 103+ | 23 " |
| Total | 104 " |

| | |
|---|---|
| History of pneumonia | 13 cases. |

though they constituted only 21.8 per cent of the total number of men vaccinated. Only 13 of the hospital admissions gave a previous history of pneumonia.

Most of the men with sharp reactions reported to the hospital within 48 hours after receiving the inoculation, but there were a small number who did not appear until the 4th or even the 5th day after injection. Apparently the slower absorption of the oily emulsion sometimes causes a delayed reaction. These men were in no instance seriously ill, though some of them were quite uncomfortable.

The symptom most often complained of was headache. Backache and general muscular aching were also very common. The symptoms described were headache (84 per cent), backache (35 per cent), general muscular aching (38 per cent), general malaise (21 per cent). chill (20 per cent), pain in chest (18 per cent), cough (12 per cent). A rise in temperature was noted in 89 per cent of the hospital admissions, but this was usually not marked. In 64 cases the temperature never rose above 102° F., and in most cases it was normal 24 or 48 hours after admission. As a rule, these patients remained in the hospital only 2 or 3 days. They rarely stayed longer than 5 days.

### *Method of Keeping Records.*

In order to keep a careful record of all cases of pneumonia that developed in camp subsequent to vaccination, the vaccination register cards were filed (1) according to organization; (2) carbon copies were filed alphabetically; (3) a special file was prepared from the soldiers' identification numbers. Whenever a case of pneumonia was reported, a search was made for the patient's name in all three files. In addition to searching the file, the patient himself was carefully questioned as to whether or not he had received the pneumonia vaccine. By this method of procedure, the percentage of error in our results was probably reduced to a very low figure.

### *Sputum Examination.*

Realizing from the outset the great importance of accurate bacteriological study on all cases of pneumonia, we made arrangements for a large supply of white mice and were able to maintain this supply

throughout the entire experiment. In this way practically every sputum examined was tested by the mouse method. In a series of 690 cases of pneumonia, there were only 17 cases in which the sputum was not examined. It had been our original intention to check every sputum, either by examination of a second specimen or by having two independent observers make examinations on the same specimen. During the height of the influenza epidemic, however, it was found impossible to maintain this method of control on every case. Of the 673 cases of pneumonia in which a sputum analysis was made, 368 were controlled by a second examination or by a second observer. The second examination was not always made on a specimen of sputum, however. In some instances it was a positive blood culture, an empyema exudate, or cultures taken directly from the lung at autopsy. In the majority of instances, the two examinations were consistent, and often where the findings were inconsistent, there was good reason to believe that we were dealing with a mixed infection. In the final classification of pneumonia cases, however, we have tried to avoid a group of mixed infections and, in some cases where two or more organisms have been found, have classified them according to the virulence of the types. For example, in a number of cases, Type IV pneumococcus was found in association with one of the fixed types of pneumococcus, and such cases were always classified as fixed types.

### *Results of Vaccination.*

The results of vaccination against pneumonia, as shown by the incidence of pneumonia in vaccinated and unvaccinated troops, are indicated in Tables I, IV, and VI. In Table I the classification of all cases of pneumonia occurring at Camp Wheeler between July 1 and October 1, 1918, is compared with a classification of all the cases of pneumonia occurring at this camp from October 1 to December 20, 1918. From these figures it will be observed (1) that the incidence of pneumonia for the 3 months July 1 to October 1 was comparatively high, in view of the season of the year; (2) that there were three times as many cases of pneumonia during the following 3 months (October 1 to December 20) due to the influenza epidemic; (3) that pneumonia

TABLE IV.

*Cases of Pneumonia at Camp Wheeler from September 21 to December 20, 1918 (Period of Experiment). Number of Men Vaccinated against Pneumonia, 13,460 (about 90 Per Cent of Strength of Camp).*

| Types. | Vaccinated. | | | | | | Unvaccinated. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of cases. | | | No. of deaths. | | | Mortality. | | No. of cases. | | | No. of deaths. | | | Mortality. | |
| | | | | | | | *per cent* | | | | | | | | *per cent* | |
| Pneumococcus, I | 7 | 32 | (9.6%) | 2 | 10 | (13.6%) | 28.5 | 31.2 | 6 | 42 | (14%) | 2 | 14 | (25.5%) | 33.3 | 33.3 |
| " II | 3* | | | 1 | | | 33.3 | | 16† | | | 6 | | | 37.5 | |
| " III | 22 | | | 7 | | | 31.8 | | 20 | | | 6 | | | 30.0 | |
| " IV | 298 | | (90.4%) | 63 | | (86.4%) | 21.1 | | 256 | | (86%) | 55 | | (74.5%) | 21.4 | |
| Hemolytic streptococcus | 5 | | | 3 | | | 60.0 | | 5 | | | 2 | | | 40.0 | |
| Non-hemolytic " | 21 | | | 2 | | | 9.5 | | 13 | | | 0 | | | 0 | |
| Friedländer's bacillus | 0 | | | 0 | | | 0 | | 1 | | | 1 | | | 100 | |
| Undetermined | 7 | | | 1 | | | 14.2 | | 10 | | | 1 | | | 10.0 | |
| Total | 363 | | | 79 | | | 21.7 | | 327 | | | 73 | | | 22.3 | |

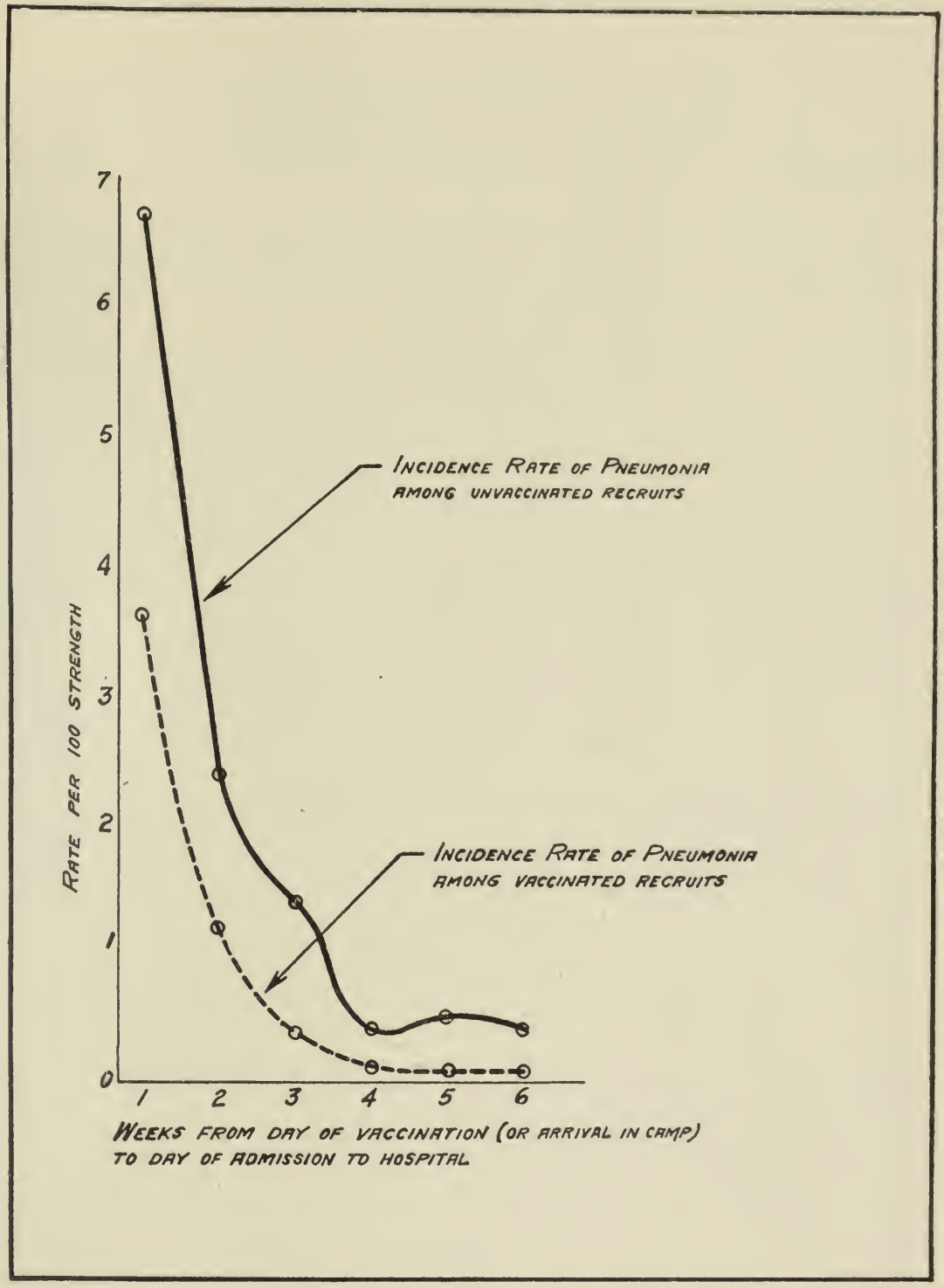
* These three cases were all atypical Type II.

† Of these sixteen cases ten were typical Type II, and six were atypical Type II.

of Types I, II, and III constituted 43.2 per cent of all the pneumococcus cases in the period from July 1 to October 1, while in the period from October 1 to December 20, Type I, II, and III pneumococci caused only 11.5 per cent of all the pneumococcus pneumonia; (4) that the mortality rate for all cases was 15.7 per cent during the summer months and 22.4 per cent during the influenza epidemic.

Table IV shows the relative incidence of pneumonia among vaccinated and unvaccinated troops during the period of the experiment, September 21 to December 20, 1918. In comparing these figures it should be borne in mind that during most of this period 80 per cent of the men were vaccinated against pneumonia. Examination of Table IV shows that, during these 3 months, there were 32 cases of pneumonia of Types I, II, and III among vaccinated men and 42 cases of pneumonia of these types among the unvaccinated; there were 298 Type IV pneumonia cases among the vaccinated men, as compared with 256 Type IV cases among the unvaccinated. In other words, 9.6 per cent of the pneumococcus cases were caused by fixed types of pneumococcus in the vaccinated group and 14 per cent were caused by the same types among the unvaccinated. Altogether, there were 363 cases of pneumonia during this period among vaccinated men and 327 among the unvaccinated. The incidence of pneumonia was about the same in the two groups, though the vaccinated group represented 80 per cent of the camp strength. The mortality rate in the vaccinated series, 21.7 per cent, was slightly lower than that for the unvaccinated series, 22.3 per cent.

These figures are not altogether encouraging, but a more careful analysis shows that other factors have to be considered before any just estimate of the value of the vaccine can be arrived at. In the first place, Text-fig. 1 shows clearly that the weekly incidence rate of pneumonia was conspicuously lower among the vaccinated than among the unvaccinated troops. It will be observed that the scope of the two rate curves is about the same between October 12 and 19, the time at which the influenza epidemic began to make itself felt at Camp Wheeler. The following week, as the vaccinated men began to develop protection, the rate for the vaccinated ceased to increase, then rapidly declined. Had these men remained unvaccinated, the pneumonia rate for this particular group would almost certainly have




TEXT-FIG. 2. Comparison of the pneumonia case rate (all types) among vaccinated and unvaccinated recruits.

reached a high peak by October 26 as did that for the unvaccinated group.

Text-fig. 2 shows that a large percentage of pneumonia cases occurring among the troops at Camp Wheeler developed among the recruits, whether vaccinated or unvaccinated, during the first 2 weeks of their residence in camp.

Colonel Whitmore in his recent Harvey lecture (5) reports some experiments with pneumococcus lipovaccine which show that immunity

TABLE V.

*Showing Development of Protective Bodies against Pneumococcus Type I in Human Serum Following Injection of 1 Cc. of Pneumococcus Lipo-Vaccine.*

| Date. | Control. | | Nov. 17 | Nov. 18 | Nov. 19 | Nov. 20 | Nov. 21 | Nov. 22 | Nov. 23 | Nov. 25 | Nov. 26 | Nov. 28 | Nov. 29 | Dec. 1 | Dec. 4 | Dec. 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days after vaccination. | | | Before. | | 1 | 2 | 3 | 4 | 5 | 7 | 8 | 10 | 11 | 13 | 16 | 32 |
| 0.000001 cc | 30 | 30 | 30 | 36 | 36 | 36 | 30 | 24 | 24 | 36 | S. | S. | S. | S. | S. | S. |
| 0.00001 cc | 30 | 36 | 30 | 24 | 30 | 24 | 30 | 30 | 30 | 36 | 48 | " | " | " | " | " |
| 0.0001 cc | 30 | X. | 22 | 24 | 22 | 24 | 24 | 30 | 30* | 30 | 30 | 30 | 30 | 30 | " | 24 |
| 0.001 cc | 30 | " | 22 | 22 | 22* | 24* | 15 | 15 | X. | 24 | 36 | 30 | 22 | 30 | 22 | 30 |
| 0.01 cc | 22 | " | X. | 22 | X. | X. | 22* | 15 | " | X. | 10 | 30* | 15 | 24 | 22 | 15 |
| 0.1 cc | X. | " | " | X. | " | " | X. | 15 | " | " | X. | X. | 15 | 15 | 22 | 15 |

Private G. vaccinated November 18, 1918, 4 p.m. Vaccine 2 mg. per cc.

Protection test December 21, 1918. 0.2 cc. of serum + multiple of minimum lethal dose of Pneumococcus Type I broth culture injected intraperitoneally into mice. Time of death noted in hours. Pneumococci found in all peritoneal exudates.

X. indicates no test; S. survival; * 0.1 cc. of serum.

Vaccine contained 0.83 mg. (about 10 billion) of each of the three fixed types, I, II, and III, pneumococcus.

against the pneumococcus does not begin to develop until the 8th day after injection of the vaccine. From that time on the immunity curve rises steadily and continues to rise for some time thereafter. Table V shows the results obtained in one of Colonel Whitmore's experiments.[1] The serum was tested before vaccination and found to have no protective power value for mice. The individual was vaccinated on November 18, and his blood taken on successive days

[1] Colonel Whitmore has kindly permitted us to publish this table.

for the purpose of studying the development of protective bodies. It will be seen from the table that there were no survivals among the mice until the 8th day after vaccination, and that from that time on the percentage of survivals increased from day to day.

With this consideration in mind, we have prepared a revised table of pneumonia cases occurring among the vaccinated men (Table VI). In this table all cases of pneumonia developing within 1 week after vaccination have been excluded. When this alteration is made, there remain only two cases of Type I pneumonia, one case of Type II (and that an atypical Type II), and five cases of Type III develop-

TABLE VI.

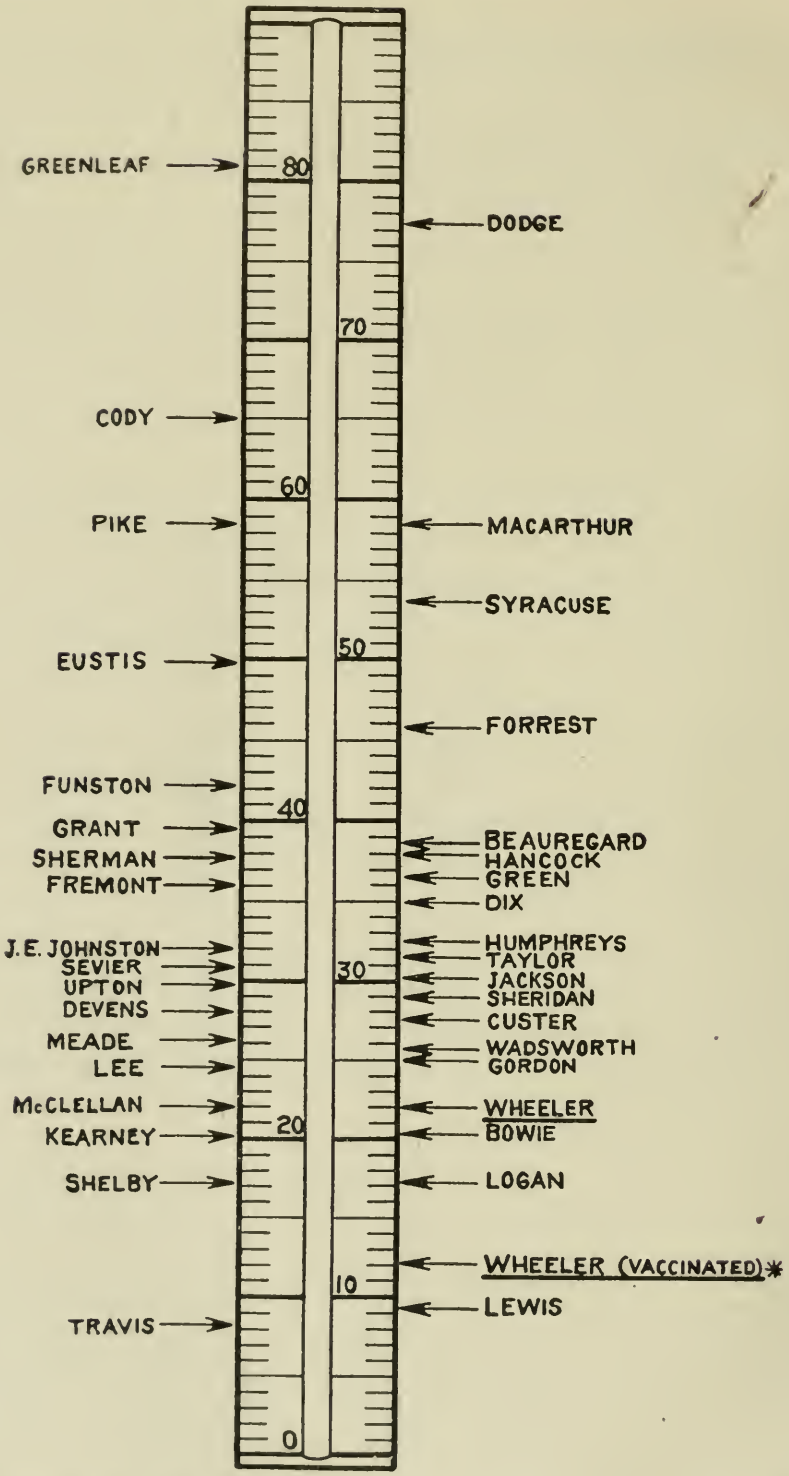
*Revised Table of Incidence of Pneumonia among Vaccinated Men from September 21 to December 20, 1918.**

| Types. | No. of cases. | | No. of deaths. | | Mortality. |
|---|---|---|---|---|---|
| | | | | | *per cent* |
| Pneumococcus, I | 2 | | 0 | | 0 |
| " II | 1† | 8 (5.8%) | 0 | 1 (6.7%) | 0 12.5 |
| " III | 5 | | 1 | | 20 |
| " IV | 124 | (94.2%) | 14 | (93.3%) | 11.2 |
| Hemolytic streptococcus | 4 | | 2 | | 50.0 |
| Non-hemolytic " | 14 | | 1 | | 14.2 |
| Undetermined | 5 | | 1 | | 20.0 |
| Total | 155 | | 19 | | 12.2 |

* In this table all cases of pneumonia developing within 1 week after inoculation have been excluded.

† This case was an atypical Type II.

ing among vaccinated men during the period of the experiment. That there were five Type III cases in this group is not so surprising when we recall the difficulty one has in immunizing animals against Type III pneumococcus. The most important and significant point, however, in connection with these eight cases of pneumonia is the fact that all eight cases followed severe attacks of influenza. Only one of the eight cases died, and this was one of the Type III group. If all pneumonia patients developing within a week after vaccination are excluded from the Type IV group of vaccinated pneumonia cases, the figure is reduced from 208 to 124 cases. 103 of the 124 cases were




TEXT-FIG. 3. Mortality rate per 100 cases of pneumonia in Army camps from September 21 to December 20, 1918.

* Rate for 155 cases of pneumonia that developed among vaccinated men 1 week or more after vaccination.

instances of secondary pneumonia and only 21 primary. There were 14 deaths among the 103 secondary pneumonia cases and no deaths in the series of 21 primary pneumonia cases.

The mortality rate for the 155 cases of pneumonia of all types that developed 1 week or later following vaccination was only 12.2 per cent. When this figure is compared with the pneumonia mortality rate for the various camps during the same period (Text-fig. 3), it will be seen that the rate for vaccinated pneumonias at Camp Wheeler was one of the lowest of any of the camps. The mortality rates for other camps were calculated from the Surgeon General's "Weekly sick report," and are accurate only in as far as the figures on incidence of pneumonia and deaths from pneumonia in the respective camps are accurate.

*Pneumonia among Recruits.*

The influenza epidemic made its first appearance at Camp Wheeler on September 30 when about 40 cases were admitted to the hospital from the Camp Stockade for General Prisoners. These men, the first to import the infection, were negro draft evaders from rural parts of Georgia who had failed to report at the time of the last draft, September 1, and who had since been rounded up as general prisoners. As soon as these draft evaders, who numbered about 150, had been mustered into service, those not in the hospital with influenza were transferred gradually to the negro companies of the Receiving Camp. Influenza was transferred to this organization with them and reached a maximum daily admission rate of 69 cases on October 4.

On October 16, 17, and 18, 1,270 negro recruits arrived in camp from rural sections of Georgia. 1,113 of these men were vaccinated for pneumonia within 24 hours after arrival. Between October 23 and 27, 4,751 white recruits arrived, approximately 3,500 from western New York State, the remainder from rural sections of Alabama. 3,382 of these men were immediately vaccinated.

During their 1st week in camp 419 negro recruits, 33 per cent of their command, developed influenza. Of these, 72 cases, or 17.2 per cent, were followed by pneumonia. These men arrived at Camp Wheeler already exposed to or infected with influenza, and were an

especially susceptible group since the epidemic had not at that time generally reached the rural counties of Georgia. 533 of the white recruits, 11.2 per cent of their strength, developed influenza during their 1st week in camp, followed by 89 cases of pneumonia, a rate of 16.7 per cent. It therefore appears that this particular group of negroes was much more susceptible to influenza than the whites; but when they had once become infected, the chances of developing pneumonia were about the same. The white recruits from New York State came from a section already generally infected with influenza.

Text-fig. 2 aims to show the relative incidence (by week) of pneumonia among vaccinated and unvaccinated recruits immediately following their arrival in camp. The bacteriological types have been left out of consideration, Pneumococcus Type IV and other organisms being included with the three types which compose the vaccine. The curves express a rate which in either case is very high for the 1st week, due to the fact that practically all of the group of recruits arrived in camp already exposed to or infected with influenza. 6.7 per cent of the unvaccinated and 3.6 per cent of the vaccinated recruits developed pneumonia within 1 week after arrival in camp. The rates for the 1st week would doubtless have been more nearly the same had it not been for the fact that those already infected with influenza were not vaccinated. As immunity does not develop until after the 8th day, it is reasonable to expect the incidence among the vaccinated to remain high during the 1st week and even during the 2nd week, as the latter group was being infected with influenza at the same time that the lipovaccine was building up resistance against pneumonia.

It is noteworthy that the weekly pneumonia rate among the vaccinated recruits remained consistently only about one-half of that for the unvaccinated. 72 unvaccinated recruits developed pneumonia during the week following vaccination, 6.7 per cent of their strength; 180 vaccinated recruits, 3.6 per cent of their strength. 53 unvaccinated recruits developed pneumonia after the 1st week, 4.9 per cent of their strength; 108 vaccinated recruits, 2.2 per cent of their strength. Furthermore, there is certainly no evidence to show that the administration of pneumococcus vaccine has in any way predisposed to an attack of pneumonia. Had such been the case the inci-

dence rate for the 1st week among the vaccinated would doubtless have exceeded that for the unvaccinated. As it was, the former was considerably inferior to the latter.

*Pneumonia among Recruits Compared with Pneumonia among Seasoned Men.*

Table VII shows that in recruits pneumonia secondary to influenza has proved more fatal than primary pneumonia, the death rate being 25.3 per cent for the former, 13.8 per cent for the latter. Moreover,

TABLE VII.

*Mortality Rates for a Series of 412 Cases of Pneumonia among Recruits. Primary Pneumonia and Pneumonia Secondary to Influenza.*

| Classification of recruits. | | Primary. | | | Secondary. | | |
|---|---|---|---|---|---|---|---|
| | | No. of cases. | No. of deaths. | Mortality. | No. of cases. | No. of deaths. | Mortality. |
| | | | | *per cent* | | | *per cent* |
| White. | Vaccinated | 6 | 1 | 16.7 | 142 | 36 | 25.3 |
| | Unvaccinated | 1 | 0 | 0.0 | 58 | 15 | 25.8 |
| Negro. | Vaccinated | 17 | 1 | 5.9 | 123 | 32 | 26.0 |
| | Unvaccinated | 5 | 2 | 40.0 | 60 | 14 | 23.3 |
| Total. | Vaccinated | 23 | 2 | 11.5 | 265 | 68 | 25.7 |
| | Unvaccinated | 6 | 2 | 33.3 | 118 | 29 | 24.6 |
| Grand total | | 29 | 4 | 13.8 | 383 | 97 | 25.3 |

in primary pneumonia it will be observed that the death rate for vaccinated cases is only one-third that for unvaccinated cases, 11.5 and 33.3 per cent respectively; whereas in secondary pneumonia the death rate is practically the same in the vaccinated and unvaccinated groups.

The seasoned men (Table VIII) show a somewhat higher death rate for pneumonia secondary to influenza than for primary pneumonia. Again, however, a marked contrast is observed in the mortality rate of primary pneumonia in vaccinated and unvaccinated men, the rate in unvaccinated (31.2 per cent) being twice that of the vaccinated (15.8 per cent). And again the death rate in secondary pneumonia

TABLE VIII.

*Mortality Rate for a Series of 168 Cases of Pneumonia among Seasoned Troops. Primary Pneumonia and Pneumonia Secondary to Influenza.*

| Classification of seasoned men. | Primary. | | | Secondary. | | |
|---|---|---|---|---|---|---|
| | No. of cases. | No. of deaths. | Mortality. | No. of cases. | No. of deaths. | Mortality. |
| | | | *per cent* | | | *per cent* |
| White. Vaccinated | 5 | 1 | 20.0 | 29 | 5 | 17.3 |
| White. Unvaccinated | 3 | 1 | 33.3 | 74 | 16 | 21.6 |
| Negro. Vaccinated | 14 | 2 | 14.3 | 13 | 1 | 7.9 |
| Negro. Unvaccinated | 13 | 4 | 30.7 | 17 | 1 | 5.9 |
| Total. Vaccinated | 19 | 3 | 15.8 | 42 | 6 | 14.3 |
| Total. Unvaccinated | 16 | 5 | 31.2 | 91 | 17 | 18.7 |
| Grand total | 35 | 8 | 22.9 | 133 | 23 | 17.3 |

TABLE IX.

*Pneumonia Rate per 1,000 for Recruits and Seasoned Men during the 3 Months of the Investigation.*

| Classification of men. | Absolute No. of men. | Absolute No. of cases of pneumonia. | Incidence per 1,000 men. |
|---|---|---|---|
| Recruits. | | | |
| White. Vaccinated | 3,832 | 148 | 38.7 |
| White. Unvaccinated | 919 | 59 | 64.0 |
| Negro. Vaccinated | 1,113 | 140 | 125.0 |
| Negro. Unvaccinated | 157 | 65 | 413.0 |
| Total vaccinated recruits | 4,945 | 288 | 58.2 |
| " unvaccinated " | 1,076 | 124 | 115.2 |
| Seasoned men. | | | |
| White. Vaccinated | 6,570 | 34 | 5.2 |
| White. Unvaccinated | 1,919 | 77 | 40.0 |
| Negro. Vaccinated | 1,828 | 27 | 14.8 |
| Negro. Unvaccinated | 383 | 30 | 78.4 |
| Total vaccinated seasoned men | 8,398 | 61 | 7.2 |
| " unvaccinated " " | 2,302 | 107 | 46.4 |

differs very little for the vaccinated and unvaccinated series. It would appear, therefore, from Tables VII and VIII that the death rate in primary pneumonia is favorably influenced by pneumococcus vaccination, but is slightly, if at all, affected by it in pneumonia secondary to influenza. The series of primary cases, however, in both recruits and seasoned men is too small to allow of definite conclusion.

Table IX is a comparison of the pneumonia rate per 1,000 men in recruits and seasoned men during the period of the experiment. It was hardly possible to determine the annual pneumonia incidence rate for each group, as the various organizations composing a group were vaccinated at different times. Table IX shows: (1) that the pneumonia rate was considerably higher among negro troops than among white troops, and that this difference was quite marked, whether the men were recruits or seasoned men; (2) that the pneumonia rate for any particular group, white or negro, recruits or seasoned men, was markedly lower for the vaccinated part of the group than for the unvaccinated part. This difference was most marked in the case of seasoned white men where the pneumonia rate for unvaccinated men was nearly seven times that for the vaccinated men.

## DISCUSSION.

It is necessary to emphasize again the fact that conditions at Camp Wheeler were not nearly so favorable for a test of pneumococcus vaccine as they had been at Camp Upton. The high incidence of pneumonia among recruits at Camp Wheeler shows how much more susceptible they are to pneumonia than seasoned men such as were vaccinated at Camp Upton. In other words, the natural immunity of recruits is much lower than that of seasoned troops, and this is particularly true of rural men, such as composed the Camp Wheeler draft. The Camp Upton division was composed chiefly of New York City men.

A combination of influenza vaccination with pneumococcus vaccination in 2,226 men was another complicating factor at Camp Wheeler, but there is no evidence that the simultaneous injection of the two interfered in any way with the effectiveness of the pneumococcus vaccine.

The influenza epidemic was, of course, the greatest disturbing element in the Camp Wheeler experiment. The type of influenza which has been recently prevalent certainly causes a marked reduction in the patient's resistance to pneumonia. It is possible that a vaccine which, under ordinary circumstances, would have given complete protection against primary lobar pneumonia would fail completely during an epidemic of such virulence as the recent influenza epidemic. Furthermore, it is reasonable to suppose that with the resisting power of the lungs at such a low ebb, even had pneumococcus infection with Types I, II, and III been prevented by means of a vaccine, some other type of pneumonia would have developed in its place. It will be recalled that at the onset of the influenza epidemic at Camp Wheeler, the proportion of Type IV pneumonia cases greatly increased even among the unvaccinated men. This may have been due to the fact that, from that time on, pneumonia at Camp Wheeler ceased almost entirely to be a primary disease, and became a complication of influenza. Under such circumstances infection by whatever virulent organism that happened to be in the mouth would have occurred, and as the Type IV pneumococcus is the type so frequently present in the normal mouth, it would naturally play a prominent part in the etiology of secondary pneumonia.

The epidemic at Camp Wheeler was about over December 1, and the effect of vaccination against pneumonia under more normal conditions could have been observed had not demobilization occurred at this time.

It is apparent that any sort of vaccination against pneumonia must, of necessity, be put to a very crucial test. Typhoid fever, for example, is a primary infection, and the typhoid bacillus rarely finds its way into the alimentary canal of healthy men. The pneumococcus, on the contrary, is frequently found in the healthy mouth and the host is therefore constantly exposed to infection at some moment when his resistance is at a low point. Moreover, pneumonia, in many of the camps at least, has been largely a secondary infection, overtaking a patient when the natural resistance has been lowered to such a degree that pulmonary infection of some kind is almost inevitable. In cases of this kind the problem is to reduce the chances of the patient's becoming infected with a highly virulent organism, so that if pneumonia does develop, the disease will run a mild course.

The present study has not been altogether satisfactory, but it has served to bring out certain points with a fair degree of definiteness. Though 80 per cent of the population at Camp Wheeler were vaccinated, almost as many cases of pneumonia developed among the unvaccinated one-fifth as occurred among the vaccinated four-fifths. If we reckon from 1 week after vaccination, the time when the individual's immunity begins to develop, only 8 cases of Type I, II, and III pneumonia occurred among the vaccinated men, and all these were secondary to severe attacks of influenza. By using the same standard, 124 cases of Type IV pneumonia developed among the vaccinated troops, and 103 of these were also secondary to influenza. Furthermore, it has been demonstrated that the weekly incidence of pneumonia (all types) among both recruits and seasoned men has been more than twice as great among the unvaccinated as among the vaccinated.

These investigations also show that the most striking results of pneumococcus vaccination are obtained with well seasoned men, where the pneumonia incidence rate per 1,000 men was only 7.2 per cent for vaccinated men and 46.4 per cent, almost seven times as great, for unvaccinated men. These findings are in complete accord with those at Camp Upton last winter where only seasoned troops were vaccinated and where the pneumonia incidence rate was ten times as great for unvaccinated men as for vaccinated. We are inclined to believe that the best time to vaccinate recruits would be 2 or 3 weeks before they come to camp.

The large number of cases of Type IV pneumonia in the present epidemic strongly emphasizes the need of a Type IV pneumococcus vaccine. Before an efficacious Type IV vaccine can be obtained, however, much careful study of the biological characteristics of this group will be necessary.

With the cessation of the war, the question naturally arises: How much use can be made of pneumococcus vaccine in civil life? Vital statistics show that pneumonia now kills more people in the United States than any other infectious disease, and the rate seems to be increasing slowly every year. Furthermore, a majority of the pneumonias seen in civilian adults is of the primary lobar type, which is the type of pneumonia most amenable to prophylactic vaccination.

If methods can be devised for still further reducing the toxicity of pneumococcus vaccine, and if active educational propaganda are instituted in its behalf, there is no reason why vaccination against pneumonia should not find a large field of usefulness in civilian as well as in Army life.

## SUMMARY.

1. 13,460 men, or about 80 per cent of the entire camp strength, were vaccinated against pneumonia with pneumococcus lipovaccine.

2. The dosage employed in all cases was 1 cc. of the lipovaccine containing approximately 10 billion each of Pneumococcus Types I, II, and III.

3. Both the local and general reactions produced by the vaccine were usually mild. Only 0.7 per cent of those who received the vaccine were sufficiently affected to need hospital care. None of these was seriously ill, and a majority of them returned to duty on the 2nd or 3rd day after admission.

4. Most of the troops inoculated were under observation for 2 or 3 months after vaccination. During this period there were 32 cases of Pneumococcus Type I, II, and III pneumonia among the vaccinated four-fifths of camp, and 42 cases of pneumonia of these types among the unvaccinated one-fifth of camp. If, however, all cases of pneumonia that developed within 1 week after vaccination are excluded from the vaccinated group, there remain only 8 cases of pneumonia produced by fixed types, and these were all secondary to severe attacks of influenza. This exclusion is justified by the fact that protective bodies do not begin to appear in the serum until the 8th day after injection of pneumococcus lipovaccine.

5. There is no evidence whatever that pneumococcus vaccine predisposes the individual even temporarily toward either pneumococcus or streptococcus pneumonia.

6. The weekly incidence rate for pneumonia (all types) among the vaccinated troops was conspicuously lower than that for the unvaccinated troops.

7. The pneumonia incidence rate per 1,000 men during the period of the experiment was twice as high for unvaccinated recruits as for vaccinated recruits, and nearly seven times as high for unvaccinated seasoned men as for vaccinated seasoned men.

8. Influenza causes a marked reduction in resistance to pneumonia even among vaccinated men. Of the 155 cases of pneumonia (all types) developing 1 week or more after vaccination, 133 were secondary to influenza.

9. The death rate for 155 cases of pneumonia (all types) that developed among vaccinated men 1 week or more after vaccination was only 12.2 per cent, whereas the death rate for 327 cases of all types that occurred among unvaccinated troops was 22.3 per cent. The death rate for primary pneumonia among vaccinated troops was 11.9 per cent. Among unvaccinated, it was 31.8 per cent, almost three times as great. On the other hand, the mortality rate in pneumonia secondary to influenza is about the same for the vaccinated and unvaccinated groups.

10. In conclusion, it must be admitted that the results of pneumococcus vaccination at Camp Wheeler have not been so striking as those obtained at Camp Upton in 1918, largely on account of the influenza epidemic; but, although influenza obscured to some extent the effect of pneumococcus vaccination at Camp Wheeler, the results are sufficiently encouraging to justify its further application in civil as well as in military life.

## BIBLIOGRAPHY.

1. Cecil, R. L., and Austin, J. H., *J. Exp. Med.*, 1918, xxviii, 19.
2. Whitmore, E. R., Fennel, E. A., and Petersen, W. F., *J. Am. Med. Assn.*, 1918, lxx, 427.
3. Le Moignic and Pinoy, *Compt. rend. Soc. biol.*, 1916, lxxix, 201, 352.
4. Achard, Ch., and Foix, Ch., *Compt. rend. Soc. biol.*, 1916, lxxix, 209.
5. Whitmore, E. R., Harvey Lecture, January 11, 1919.

# THE ACTION OF STROPHANTHIN ON THE LIVING CAT'S HEART.*

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Plates 23 to 25.

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## INTRODUCTION AND HISTORICAL REVIEW.

A review of the pharmacological literature concerning the action of the digitalis bodies reveals a complicated problem and conflicting views. Many difficulties in the way of clear understanding have resulted from the different conditions under which similar phases of this question were investigated, from the impossibility of translating the results of a study of one species to another, and from attempting to transfer the results of the study of one of the digitalis glucosides into terms of another. A prime difficulty consists in the fact that the fate of these drugs within the body is not understood, in respect either to their internal catabolism or to their excretion. This is true partly because much of the experimental work has been done on isolated frogs' hearts perfused in various ways. The interpretation of results from such experiments must be made carefully because they provide no opportunity for essential studies, such as excretion by the kidneys, combination or destruction of the drugs by other tissues, or for observing the influence of the extracardiac nervous control. For these reasons additional knowledge that will help to clarify any part of this problem is of importance, especially if it improves the technique of the clinical administration of digitalis.

*Importance of the Concentration of Digitalis and of the Total Amount.*—A review of the literature of this subject shows that one of the important problems relating to the digitalis glucosides concerns their mode of action. Does this depend on a physical or a chemical process? Many experiments on isolated frogs' hearts have given evidence that standstill of the ventricle is obtained in the same length of time even when the amount of the perfusion fluid is altered if the concentration of the active principle is kept constant. If the total amount of perfusion fluid is constant, however, the time for standstill will be inversely proportional to the concentration. Straub (1) and later Holste (2), using pure

* This work was done under tenure of a William O. Moseley Travelling Fellowship from Harvard University.

strophanthin, came to the conclusion that the action depended on the concentration to which the heart was exposed and not on the total amount of the drug. Grünwald (3), using digitalin and isolated frogs' hearts, found that although the concentration of the drug was the most important factor, the total amount of the solution and therefore of the drug, was of considerable influence in experiments where low dilutions were used. Straub (4) ascribed the discrepancy in Grünwald's results to the fact that while the latter used digitalin, he used pure strophanthin. Weizsäcker (5) agreed with Straub that although the amount of fluid was quite important in low dilutions of digitalin, the amount had no effect on the action of strophanthin. The lowest dilution of strophanthin he used, however, was 0.00025 per cent. Clark (6) and Issekutz (7) found marked differences with digitoxin and only slight, but definite ones with strophanthin when equal concentrations but different amounts were compared. The importance of the problem as to whether digitalis acts because of a certain concentration or because of a certain total amount is appreciated when one realizes that clinically the drug is given, neither to produce standstill of the heart, nor even to produce toxic effects, but rather in amounts so small as to be comparable with those low dilutions in which Grünwald, Clark, and Issekutz found that the total amount of the drug played some part. It is reasonable to suppose that when the drugs are used clinically in such small amounts and in such low dilutions the small amount taken up by the heart and perhaps by other tissues of the body would be sufficient definitely to alter the remaining concentration in the blood stream.

*Influence of Temperature and Heart Rate.*—There are many factors that influence the effects of the digitalis bodies on the heart. The effect of temperature on the rapidity of digitalis action has been thoroughly studied. Higher temperatures uniformly increase this. Trendelenburg (8) showed that lowering the temperature decreased the effect of strophanthin on perfused frogs' hearts. The difference between given changes in temperature he found to be greater with more concentrated solutions and at the lower temperatures. Gunn (9) found that the effect of strophanthin on perfused rabbits' hearts was more prompt at higher temperatures and thought that this was due to the increased coronary circulation and therefore greater exposure of the heart to the drug. Sollmann, Mendenhall, and Stingel (10) found that the toxicity of ouabain for intact frogs or their isolated hearts increased with the temperature, that this increase was greater per degree at the lower than at the higher temperatures, and that the increase was greater than could be accounted for by the increase in heart rate, which in itself enhances the action of the drug. Weizsäcker (11), Clark (12), and Issekutz (7) noted a more rapid action of the digitalis bodies on frogs' hearts when the rate of contraction as controlled by single induction shocks was increased. Weizsäcker (5) distinguished between the time it took for digitalin to show its first effect on the heart by an increase in the strength of the contractions (*Giftbindung*) and the time to produce standstill (*Giftwirkung*), and observed that the ventricular rate influenced the latter but not the former. Pittenger and Vanderkleed (13),

working with the effects of tincture and fluid extract of digitalis on gold fish, found that although the weight of the fish was not important in determining the time to produce death by a certain concentration of digitalis, changes in temperature made striking differences.

*Influence of the General Condition of the Heart and of the Pressure of the Perfusion Fluid.*—The general condition of the heart greatly influences the effect produced on it by a given amount of digitalis. Clark (12) showed that strophanthin had a more marked and more rapid effect on perfused hearts if the frog was previously in poor health, or if the heart had been long perfused. Williams (14) and later Krailsheimer (15) found that the degree of pressure to which the frog's heart is subjected in perfusion experiments affects the action of digitalin and helleborein. Clinically it is known that although the mechanism of action of the drug may be the same, the influence of digitalis on diseased hearts may result in a different effect than on normal hearts.

*Influence of the Character of the Perfusion Fluid on the Type of Standstill.*—There has been some difference of opinion as to the factors that determine the position (systole or diastole) in which a heart will stop under the intoxication of digitalis. The location where the solution is applied is important in this connection. Jacobj (16) and Wybauw (17) found that if the digitalis substance is applied to the outside diastolic standstill results, while if it is applied to the inside systolic arrest occurs. The use of blood serum in the perfusion fluid also alters the subsequent effects of digitalis. Cushny and Gunn (18), for instance, observed that the addition either of homologous or heterologous serum to Ringer solution in perfusing rabbits' hearts caused first a stage of stimulation and later a period of depression in the strength of contraction. The hearts could be restored to a normal condition by returning to the use of Ringer fluid. Gröber (19), experimenting with strophanthidin obtained by Fraser (20) from *strophanthus hispidus*, noticed that the amount of the drug used influenced the result, for whereas 0.8 mg. in 1,000 cc. of perfusion fluid arrested a cat's heart in systole, 0.5 mg. stopped it in diastole. The perfusion fluid consisted of one part of defibrinated calf blood and two parts of Ringer solution. Clark (12) found that dilute concentrations are apt to produce diastolic arrest, and more concentrated ones, systolic arrest. The effect is more marked if the heart is already in a hypodynamic condition, due to prolonged perfusion. Werschinin (21), using Williams' (14) apparatus, concluded that 0.05 mg. of strophanthin (Boehringer) in 50 cc. of Ringer solution or normal saline, arrested frogs' hearts in systole, but lower dilutions in diastole. He added that the presence of one-third the volume of rabbit blood cells or serum would enable the lower dilutions to produce systolic arrest. In this work doubt remains as to whether the pressure in the perfusion system might not have played a part in producing diastolic standstill. Clark (22), on the contrary, found that defibrinated frog blood distinctly diminished the activity of strophanthin on perfused hearts, while the serum alone had no such effect. He thought that the red blood cells absorbed small amounts of the drug.

*Influence of Inorganic Salts.*—The inorganic salts influence the effects produced by digitalis. A perfusion fluid not containing calcium requires twice the amount of digitoxin to produce standstill that is necessary with the ordinary amount of calcium, according to Clark (6). He also found that the digitalis effect is slightly increased by the absence of potassium salts. Conversely, Burridge (23) found that the presence of digitoxin increased the sensitiveness of the heart to calcium.

*Fate of Digitalis Bodies in the Organism.*—The study of the fate of digitalis glucosides within the body, *i.e.* their combination, destruction, and the method of their excretion, is still incomplete. Cloetta and Fischer (24) gave 1 mg. of digitoxin to each of ten frogs; at the end of 1 hour all the hearts were arrested. In extracting the ten hearts no digitoxin was found by a method that could detect 0.04 mg. None was found in the hearts of twenty additional frogs although an emulsion of the rest of the bodies, excluding the skins, yielded one-third of the digitoxin injected. Nor was any found in the hearts of rabbits and dogs killed with the drug. But when emulsions of heart muscle were incubated with digitoxin at 37°C. for 4 hours, twice as much digitoxin could be recovered from the sediment, that is from the heart tissue, as from the supernatant fluid. On incubating liver a similar result was noticed. Incubations of this nature therefore did not destroy the drug, for practically the entire amount of digitoxin could be recovered from dog's heart muscle, liver, or brain. In similar incubation experiments of frog's heart, muscle, and liver, Clark (22) likewise found that strophanthin was neither destroyed nor combined with unincubated tissue. The behavior of digitalis bodies under the influence of heart muscle in the living animal and of incubated heart muscle accordingly differs. Cloetta and Fischer also examined the urine and noticed that varying amounts of the drug could be detected; in one experiment, 0.3 mg. of digitoxin, for instance, was recovered 4 hours after 3 mg. had been injected into the vein of a rabbit; in another experiment, 0.8 mg. was found in the urine during 4 days on which 4.9 mg. were injected subcutaneously. In still another experiment, 1 hour and 20 minutes following a large intravenous dose of 5 mg. to a dog, they found none in the heart, 0.8 mg. in the liver, and 3 mg. in the entire blood. Clark (22) in his experiments found part of the injected drug in the blood. He gave 0.02 mg. of strophanthin to each of six frogs subcutaneously; all died in $\frac{1}{2}$ hour, and he found about 0.01 mg., or 8 per cent, in the entire blood. In what physical or chemical state it circulates in the blood is not known, but that it is active has been proved by experiment. Clark (22) has shown that small amounts of strophanthin could be found in the blood of grass snakes 16 hours after subcutaneous injection. The whole blood of frogs, he found, diminished the action of strophanthin. Serum alone or washed corpuscles alone had no such effect. He thought that the red blood cells absorb strophanthin in small amounts but only in the presence of serum. Rabbit red cells, on the other hand, do not absorb strophanthin. But in the absence of satisfactory methods for detecting the digitalis bodies, their

presence and the duration of their action cannot be accurately known. Hatcher (25) tested out nine cats and found that after intravenous injection of 0.3 mg. of ouabain, one showed 32 per cent in the blood stream, one 45 per cent, and seven none at all. Similarly after the injection of 5 mg. of digitoxin in two different cats, none was found in the blood in 4 and 5 minutes.

These experiments show that part of the drug remains in the blood stream for a while at least, that a portion is excreted unchanged by the kidney, and that the fate of the rest is uncertain. The heart, therefore, appears either to utilize very small amounts, or to render the drug unrecognizable after it has been acted upon. Hatcher (26) made similar studies and found that in alcoholic extracts of all the tissue of the rat made 5 minutes after subcutaneous injection, strophanthin could be recovered almost entirely. But if the rat was allowed to live a day, only a portion could be found in the gastrointestinal tract, and none in the other tissues. The tissues of the rat, which are highly tolerant to digitalis, destroy the drug, and part of the drug is reexcreted into the gastrointestinal tract. Hatcher and Bailey (27) also showed that in the duodenal loop of a dog, without producing toxic effects, an amount of strophanthin could be destroyed equal to 10 times the minimum lethal dose administered intravenously. This then is the evidence in favor of the view that animal tissue destroys digitalis bodies.

*Perfused Hearts.*—The experiments of Cloetta and Fischer, Clark, and Hatcher accordingly show that digitalis substances cannot be recovered from living heart muscle, but can be found if the muscle is incubated. Some indication of the amount of digitalis that is taken up and destroyed by the heart in producing standstill may be obtained from perfusion experiments. Straub (1) perfused the heart of a number of frogs (average weight 68 gm.) with the same solution until it was arrested. Although unable to extract any digitalis from the poisoned hearts, he concluded that a certain amount, probably less than 0.0002 mg., was used up. Clark (6) by a similar technique determined that each frog's heart used up about 0.0013 mg. of digitoxin and 0.00008 mg. of strophanthin. Grünwald (3) and Weizsäcker (5) found that to accomplish this result the equivalent amounts of digitalin were 0.05 to 0.1 mg. Weizsäcker (5) made the important observation that the amount of digitalin that combines with the heart is the same, whether the heart is arrested rapidly by a concentrated solution, or more slowly by a weaker solution. If amounts no larger than these suffice to arrest the heart, the amount of the poison in the perfusion fluid must be several times greater than the amount that is taken up by the heart; *i.e.*, in order to be lethal the minimum amount of strophanthin contained in 2 cc. of fluid must be 0.001 mg. But of this amount of strophanthin, the heart, as far as the experiments show anything, takes up somewhere between 0.0002 mg. and 0.00008 mg. (average 0.00014 mg.). In other words, a much greater amount is necessary than is actually made use of.

Issekutz (7) calculated the concentration of strophanthin within the heart by weighing the heart and determining how much strophanthin was lost from the

perfusion fluid. He found in this way that the concentration was 40 times as great inside the heart as in the perfusion fluid and 15 to 20 times as great for digitoxin. Weizsäcker (5) had previously calculated in a similar way that the concentration of strophanthin within the heart muscle was just equal to that in the fluid, while that of digitalin was 25 times as great within the heart muscle as in the perfusion fluid. But careful analysis of his figures shows an error in his calculation for he did not compare the lowest dilution of strophanthin in the perfusion fluid that can cause standstill with the concentration in the heart. This would bring the figure to about 20 and would then agree with the findings of Issekutz. It must be recognized in this connection that when a diminution of the drug is found in the perfusion fluid, it need not be in the heart, as has been mentioned above. It is more probable that it is destroyed by the heart and is no longer recognizable, and that the concentration of the active principle in the perfusion fluid is slightly diminished but always greater than in the heart.

There are facts that point to a selective action of the digitalis bodies on the heart, although other body tissues seem to be affected by the drug. Clark (22) found, for instance, that 0.0005 mg. per cc. of strophanthin was required to produce standstill of the heart, 0.1 mg. per cc. to produce vasoconstriction of the leg or contraction of an isolated stomach ring, and 1 mg. to kill voluntary muscle. And Schliomensun (28) found that alcoholic phosphatids, which possess an especial capacity for combining chemically with the digitalins, could be isolated from the hearts of the human cadaver and of the dog while the corresponding fractions obtained from the skeletal muscles had no such affinity.

*Action by Physical or Chemical Processes.*—The apparent dependence of these drugs on their concentration has led to the view that their mode of action is a physical one at the surface of the muscle. There are facts, however, that point rather to a chemical change in the heart. Straub (1) showed in perfusion experiments that, if strophanthin solutions were removed and the heart was washed, it revived; recovery did not take place if washing was delayed too long. In the latter event the action was not reversible; *i.e.*, the action of the drug is physical to a certain point only. Issekutz (7) similarly found that the heart frequently could not be revived, even if washed before showing a toxic effect. Clark (6) perfused frogs' hearts with 3 cc. of a solution containing 0.01 mg. of digitoxin. He found, like Straub, that if the heart was washed in 8 minutes, it died subsequently in systole, but if washed in 5 minutes, standstill was prevented.

The analogies which have been made use of in this discussion are numerous. It is impossible, however, to interchange apparent equivalents in a problem as complex as that of the action of the digitalis glucosides. The following facts illustrate this point. The subcutaneous minimum lethal dose of strophanthin is 30 times greater for a rat than for a rabbit and 20 to 40 times the concentration is needed in the perfusion fluid to arrest the isolated heart. On the other hand, while the intact grass snake is 30 times more tolerant to a subcutaneous injection of strophanthin than is the frog, the isolated heart is 1,000 times more

tolerant. Another instance in which simple substitution would lead to error occurs in determining the relative toxicity of the substances for a certain concentration and inferring that the same relation holds true for other concentrations. Issekutz (7), for instance, shows that 5 cc. of 0.1 per cent digipuratum is equivalent to 0.001 per cent strophanthin, *i.e.* a ratio of 1 : 100, but 5 cc. of 0.03 per cent digipuratum is equivalent to 0.0000625 per cent strophanthin, a ratio of 1 : 500.

The foregoing review indicates how difficult it is to resolve some of these factors. The more important ones are temperature, rate, the general condition of the heart, the pressure of the perfusion system, and the organic and inorganic constituents in perfusion fluids. Animals differ widely; and their reactions are not constant when the mode of administration is changed or when different glucosides are compared. The experiments often yield contradictory results. Careful analyses sometimes uncover the cause. Occasionally, however, the lack of harmony cannot be explained. The absence of a chemical test for digitalis renders it impossible to make any but general statements about excretion, destruction, and combination of the drug by the living organism. But this review indicates in part wherein the difficulties of the digitalis problem lie. They are comprised principally in the diversity of the methods employed, and in the failure to appreciate that results obtained so variously can scarcely serve as the basis of satisfactory generalization. There is no intention to obscure the value in themselves of the important contributions which have so far been made. But with the means now at our disposal and as the result of the experience now accumulated it becomes important that certain requirements be observed in the plan of the experiments in order that the results may be useful in laying the foundations for clinical practice. In the experiments now reported cats have accordingly been employed because of their relatively uniform reaction to the digitalis bodies and because the same animal can be used for a number of separate experiments. Repeated use in this way made it possible to avoid the danger of lack of uniform results due to varying susceptibility even in animals of the same species. The technique of electrocardiographic registration permitted the performance of the experiment so that operative procedures on the heart itself were not undertaken and disturbances from this source were avoided. This technique in addition afforded the opportunity to study in detail the influence of time on the results of administration.

## *Method.*

Cats were selected in preference to other laboratory animals because their reaction to digitalis is more like that of the human subject. Crystalline g-strophanthin was used because of its chemical purity and to avoid the confusion from interaction of the principal glucosides if the whole digitalis leaf is used. A 1 per cent alcoholic solution was prepared and kept, and from this a 0.001 per

cent solution was made up every few days. This was used in the intravenous injections. There was no indication that the stock solution deteriorated during the period of the experiments. The solutions were kept sterile. The cats were etherized during the operative procedure. Afterward as little ether was used as was necessary to keep the animal sufficiently quiet for taking satisfactory electrocardiograms. But in consequence cats were occasionally lost by an overdose of ether, although it was always possible to distinguish by the electrocardiograms an ether death from one produced by strophanthin. During some experiments no additional ether was given after the injections were started. The strophanthin solution flowed from a graduated burette into the right saphenous vein.

*Use of the Electrocardiograph to Detect the Toxic Effect.*—The string of the galvanometer (Edelmann model) was connected with the right fore and left hind leg (Lead II). Electrocardiograms were taken before the injection of the drug and frequently during the course of the experiment. It was observed by Halsey (29) that if 40 to 60 per cent of the minimum lethal dose[1] of strophanthin is given intravenously extrasystoles appear. He fixed no time interval during which he gave strophanthin, although he states that 30 per cent was given in about 15 minutes. He gave the rest at irregular intervals. It is, in fact, impossible to find in any previous work determinations of what portion of the minimum lethal dose, given in a definite interval of time, is required to produce extrasystoles. The smallest amount required to cause ectopic premature beats we propose to call the "minimum toxic dose." The only reliable method of determining the minimum toxic dose is electrocardiographic. This method excludes the possibility of misinterpreting the nature of the irregularity. It also dispenses with the necessity of exposing the heart or disturbing the animal. It is

[1] The minimum lethal dose of crystalline strophanthin for cats as determined by Hatcher and Brody (Hatcher, R. A., and Brody, J. G., *Am. J. Pharm.*, 1910, lxxxii, 360) is 0.1 mg. per kilo of body weight if given intravenously and almost continuously over a period of 1½ hours. This was confirmed by Jamieson (Jamieson, R. A., *J. Exp. Med.*, 1915, xxii, 629), who used the same active principle as that in the experiments reported here.

more reliable to use the occurrence of ventricular ectopic beats as the index of toxic action than the vagal effects, such as slowing of the heart rate, lengthening of the auriculoventricular (P–R) interval, or the development of exaggerated sinus arrhythmia.[2] Electrocardiograms of typical toxic reaction with subsequent death in ventricular fibrillation are shown in Fig. 1. In a very few instances the toxic effect was similar to those described by Cohn (30) and Christian (31) as resulting from digitalis intoxication in man. The form of the ventricular complex continually changes; an example of this reaction is shown in Fig. 2. Injections were made every 6 minutes and frequently, on approaching the toxic dose, the interval was diminished to 3 minutes. The method of interrupted administration introduces an error of from 3 to 6 per cent in determining the minimum toxic dose, for by giving injections every 6 minutes at the rate of 60 per cent of the minimum lethal dose in an hour, 6 per cent of the minimum lethal dose is given with each injection. The final 6 per cent that is administered, which produces the toxic effect, might have been excessive by 5 per cent, for an additional 1 per cent might have produced the same toxic effect. This error cannot be avoided by giving an absolutely continuous injection, for in that case, the amount of drug given during the last few minutes before the toxic effect appears might have been unnecessary.

*Frequency of Spontaneous Nodal Rhythm in Cats.*—In the interpretation of cardiac irregularities in cats, attention is called to the great frequency of spontaneous nodal rhythm (Fig. 3). It would be almost impossible to recognize this alteration in the heart's mechanism by any means other than electrocardiographic. Of seventeen animals (Table I), nine showed nodal rhythm one or more times during the various experiments. Some cats showed it several times, others only once or twice. Eight showed it before strophanthin was given in that particular experiment. Some of them also showed it during the early part of the administration, when very small amounts had been given, one after 8 per cent of

[2] Although more reliable, we do not recommend the occurrence of ventricular ectopic beats as the sign of toxic action in clinical medicine, as earlier signs are available.

TABLE I.

| Experiment No. | Date. | Weight. | Rate of injection. | | Minimum toxic dose (amount to produce toxic effect). | Per cent of minimum lethal dose required to produce effect calculated from. | | Time for effect. | Type of effect.* | Spontaneous irregularity. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Per cent of minimum lethal dose. | Time. | | Original weight. | Actual weight. | | | |
| | *1917* | *kg.* | | | *cc.* | | | | | |
| 1 a | Jan. 8 | 3.00 | 40 | 1 hr. | Leak in cannula. | ? | ? | 104 min. | V. E. C. | |
| b | " 10 | 2.65 | 40 | 2 hrs. | 13.8 | 46 | 52 | 133 " | " | N. R. |
| c | " 12 | 2.40 | 40 | ½ hr. | 8.4 | 28 | 35 | 23 " | " | |
| d | " 16 | 2.05 | 40 | ¼ " | 15.2 | 50 | 74 | 20 " | " | |
| 2 a | Jan. 8 | 4.05 | 40 | 1 hr. | Leak in cannula. | ? | ? | 134 min. | V. E. C. | |
| b | " 11 | 3.65 | 40 | 2 hrs. | 16.0 | 40 | 43 | 117 " | F. P. V. C. | |
| c | " 12 | 3.50 | 40 | ½ hr. | 16.0 | 40 | 46 | 28 " | " | |
| d | " 16 | 3.45 | 40 | ¼ " | 25.5 | 62 | 70 | 24 " | V. E. C. | N. R. |
| 3 a | Jan. 9 | 3.35 | 40 | 1 hr. | 13.4 | 40 | 40 | 56 min. | V. E. C. | N. R. |
| b | " 11 | 3.35 | 40 | 2 hrs. | 15.6 | 47 | 47 | 141 " | " | " |
| 4 a | Jan. 16 | 3.65 | 40 | 1 hr. | 22.0 | 60 | 60 | 95 min. | F. P. V. C. | N. R. |
| b | " 20 | 3.40 | 40 | ¼ " | 16.8 | 46 | 49 | 20 " | C. V. C. | " |
| 5 a | Jan. 18 | 3.00 | 40 | 1 hr. | 18.0 | 60 | 60 | 88 min. | V. E. C. | |
| b | " 22 | 2.70 | 40 | ¼ " | 19.2 | 64 | 71 | 23 " | " | |
| c | " 26 | 2.65 | 40 | 2 hrs. | 12.0 | 40 | 45 | 117 " | F. P. V. C. | N. R. |
| 6 a | Jan. 23 | 3.00 | 50 | 1 min. | 15.0 | 50 | 50 | 13 min. | Tracings lost. | |
| b | " 29 | 2.80 | 50 | 1 hr. | 10.5 | 35 | 38 | 38 " | C. V. C. | |
| c | Feb. 2 | 2.35 | 50 | 2 hrs. | 9.0 | 30 | 38 | 70 " | F. P. V. C. | |
| 7 a | Jan. 24 | 2.25 | 60 | 1 min. | 13.5 | 60 | 60 | ∞ | 0 | |
| b | " 30 | 2.00 | 60 | 1 hr. | 11.7 | 52 | 59 | 49 min. | F. P. V. C. | |
| c | Feb. 5 | 1.65 | 52 | 1 min. | 11.7 | 52 | 71 | 12 " | C. V. C. | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 8 a | Jan. 25 | 2.95 | 60 | 1 hr. | 18.5 | 63 | 63 | 76 min. | F. P. V. C. | |
| b | " 30 | 2.50 | 60 | ¼ " | 15.2 | 52 | 61 | 13 " | V. E. C. | |
| c | Feb. 6 | 2.00 | 60 | 2 hrs. | 11.7 | 40 | 59 | 96 " | F. P. V. C. | N. R. |
| 9 a | Feb. 7 | 4.40 | 60 | ¼ hr. | 19.3 | 44 | 44 | 11 min. | C. V. C. | |
| b | " 13 | 3.45 | 60 | 2 hrs. | 20.8 | 47 | 60 | 91 " | O. P. V. C. | Bigeminy. |
| c | " 19 | 3.15 | 60 | 1 hr. | 15.6 | 35 | 49 | 35 " | V. E. C. | |
| d | " 23 | 2.90 | 60 | 4 hrs. | 18.9 | 43 | 65 | 173 " | F. P. V. C. | |
| 10 a | Feb. 8 | 3.20 | 60 | 2 hrs. | 23.0 | 72 | 72 | 143 min. | F. P. V. C. | |
| b | " 13 | 2.90 | 60 | ½ hr. | 22.8 | 71 | 78 | 36 " | ? | |
| 11 a | Feb. 9 | 3.25 | 60 | 2 hrs. | 23.3 | 72 | 72 | 143 min. | O. P. V. C. | |
| b | " 14 | 2.90 | 60 | ½ hr. | 20.6 | 64 | 71 | 35 " | V. E. C. | |
| 12 a | Feb. 14 | 3.00 | 60 | ¼ hr. | 14.4 | 48 | 48 | 9 min. | V. E. C. | N. R. |
| b | " 20 | 2.45 | 60 | ½ " | 10.8 | 36 | 45 | 17 " | " | " |
| c | " 24 | 2.35 | 60 | 1 " | 12.6 | 42 | 54 | 41 " | " | " |
| d | " 28 | 2.15 | 60 | 4 hrs. | 10.8 | 36 | 50 | 122 " | " | " |
| 13 a | Feb. 15 | 2.65 | 60 | 1 hr. | 6.4 | 24 | 24 | 23 min. | O. P. V. C. | |
| b | " 20 | 2.25 | 60 | ¼ " | 6.4 | 24 | 28 | 5 " | " | N. R. |
| 14 a | Feb. 16 | 3.40 | 60 | 4 hrs. | 18.0 | 53 | 53 | 214 min. | F. P. V. C. | |
| b | " 21 | 2.95 | 60 | 1 hr. | 18.0 | 53 | 61 | 48 " | V. E. C. | N. R. |
| c | " 26 | 2.50 | 60 | ¼ " | 21.3 | 63 | 85 | 17 " | F. P. V. C. | |
| 15 a | Mar. 2 | 4.70 | 35 | 2 min. | 16.4 | 35 | 35 | 16 min. | V. E. C. | |
| b | " 7 | 4.55 | 60 | 1 hr. | 16.8 | 36 | 37 | 38 " | F. P. V. C. | |
| 16 a | Mar. 5 | 3.15 | 30 | 1 min. | 13.1 | 42 | 42 | 42 min. | C. V. C. | |
| | | | 60 | 2 hrs. | | | | | | |
| b | " 9 | 2.75 | 30 | 1 hr. | 15.2 | 48 | 55 | 45 " | F. P. V. C. | |
| 17 a | Mar. 8 | 2.85 | 30 | 1 min. | 14.4 | 51 | 51 | 83 min. | V. E. C. | |
| | | | 60 | 2 hrs. | | | | | | |
| b | " 19 | 2.45 | 39 | 1 min. | 11.0 | 39 | 45 | 9 " | F. P. V. C. | |

* V. E. C. indicates ventricular ectopic tachycardia; F. P. V. C., frequent premature ventricular contractions; O. P. V. C., occasional premature ventricular contractions; C. V. C., changing ventricular complexes; N. R., nodal rhythm.

the minimum lethal dose had been injected. Two others, No. 9 b and one not included in Table I, showed spontaneous bigeminy, every other beat being a premature ventricular systole, before any strophanthin was given. Three cats had nodal rhythm in the first experiments (marked "a" in Table I) on the respective animals. There is no reason to believe that the cats had taken any drug that might have caused this rhythm. None of them had previously been experimented upon. The high incidence of spontaneous nodal rhythm is pointed out because it may have a bearing on experiments concerned with this arrhythmia as in the work of Lewis, White, and Meakins (32).

*Independence of the Minimum Toxic Dose of the Speed of Administration of the Drug.*—In the experiments to be described we compare the effects of rapid with slow administration in the same animal. The method is designed to yield information on the problem of whether strophanthin acts by means of its concentration or by virtue of the total amount present. The volume of the blood remains constant. By injecting it rapidly strophanthin is distributed in the circulation quickly, and the heart, therefore, is immediately exposed to a high concentration; when the injections are slow, the heart is exposed to a lower concentration for a given length of time, for a smaller amount of the active principle is contained in the same volume of blood. Thus the rate of injection determines the concentration in the blood stream. Some cats served for four separate experiments, others for three, and some for only two. The method of administration was changed from cat to cat; some received the rapid injection first and later the slow one. The reverse method was employed in others. The original minimum lethal dose was calculated on the original weight, and in later experiments the same dose was used, although the weight had diminished. This will be discussed below. In this way one could determine whether the animal's later condition as a result of the action of the drug, or the long experimentation, or the administration of ether, altered the amount of the drug required to produce the same toxic effect. There was no indication that any striking change in the animal's susceptibility to the drug took place. In the first experiments the solution (0.001 per cent)

was given at such a rate that 40 per cent of the minimum lethal dose was injected in an hour. The same cat, usually 4, though sometimes more and occasionally fewer days later, when free of the drug was subjected to the same procedure but the rate of injection was 40 per cent in 2 hours, or 40 per cent in $\frac{1}{2}$ hour, etc. The cat was considered free from the effects of the drug because Hatcher (33) showed that 24 to 48 hours following a toxic but nonlethal intravenous injection of strophanthin, a similar dose is required to produce a similar result. In the later experiments 60 per cent of the minimum lethal dose was given, because it caused toxic effects more regularly. The injections were stopped when the toxic effect occurred. This could usually be detected by watching the shadow of the galvanometer string, because of the abnormal form of the ectopic beat, and the occurrence of an irregularity. Occasionally it was difficult to be certain whether a toxic effect was produced; then electrocardiograms were taken frequently. When the films were developed, the intoxicating dose was estimated.

## EXPERIMENTAL.

The experiments are divided into three groups: those in which was studied the influence of the speed of injection on the amount of strophanthin required to produce a toxic effect; those in which single injections were made to obtain the minimum toxic and lethal dose and to compare this with the amount required when the rate of injection is slow; and transfusion experiments made to obtain additional information on the concentration of the drug in the blood.

*Group 1.*—Group 1 (Table I) contains seventeen experiments. In these was studied the influence of the speed of injection on the amount of strophanthin required to produce a toxic effect. The animals frequently lost weight. Whether this resulted from loss of appetite, or from prolonged etherization and the operative procedure, or from strophanthin intoxication is not known. The animal usually ate nothing after the operation and very little the next day. On the 4th day it was usually operated upon again. The loss in weight was disturbing in calculating the minimum lethal

dose. It seemed correct, however, to base the minimum lethal dose on the original, rather than on the actual weight (both of which were ascertained), for it is not likely that the heart lost weight during these experiments as rapidly as the whole body.[3]

There are (Table I) distinct individual variations among cats in susceptibility to toxic doses of strophanthin. It required, for instance, 72 per cent of the minimum lethal dose, given at the rate of 60 per cent in 2 hours, to produce toxic effects in Cats 10 a and 11 a, while only 24 per cent, at the rate of 60 per cent in 1 hour, was required in Cat 13 a. The difference in rate of administra-

TABLE II.

| Average. | Per cent of minimum lethal dose required to produce toxic effect, calculated from. | |
|---|---|---|
| | Original weight. | Actual weight. |
| Of all in Table I | 47.4 | 53.7 |
| " first injections ("a" in Table I) | 51.6 | 51.6 |
| " all subsequent injections | 48.7 | 54.8 |
| " last injections | 46.5 | 56.9 |
| " 1 hr. first injections | 49.4 | 49.4 |
| " ¼ " injections | 50.3 | 58.9 |
| " ½ " " | 47.8 | 55.0 |
| " 1 " " | 45.5 | 49.5 |
| " 2 hrs. " | 45.3 | 52.0 |
| " 4 " " | 44.0 | 56.0 |

tion will be shown not to influence the minimum toxic dose. Other animals required amounts between these limits. The significant point is that in the second, third, and fourth injections in the same animal, although slight variations in the minimum toxic dose occur, they do not depend on the speed of administration. This conclusion is warranted from the average of the experiments (Table II) and from an analysis of the individual ones.

The average minimum toxic dose of strophanthin in all the experiments (Table II) was 47.4 per cent of the minimum lethal dose,

[3] In a fasting cat the heart lost only 2.6 per cent of its weight, while the liver lost 53.7 per cent, and the whole body over 1,000 gm. (Howell, W. H., A text book of physiology for medical students and physicians, Philadelphia and London, 5th edition, 1913, 914).

if the original weight, and 53.7 per cent if the actual weight before each experiment is used as the basis of calculation. The second figure is higher than the first because of the animal's loss in weight. The average of all first experiments before loss of weight has occurred is 51.6 per cent. The average in the first experiments in which injections were given in an hour is 49.4 per cent. In these the influence of the loss of weight due to experimentation is entirely avoided. On the whole, there are no serious discrepancies in the various averages calculated in Table II. They show that the speed of injection does not influence the minimum toxic dose. The average minimum lethal dose in the short 15 minute experiments was 50.3 per cent; in the longer 4 hour ones 44.0 per cent. In those of intermediate length the percentages increased as one might expect. One might infer from the figures based on the original weights that the slower method requires slightly less strophanthin than the rapid, but the difference is probably not significant. An explanation for it may exist in the fact that in the slower method the dose given just before the toxic effect is produced, has acted a longer time on the heart; while in the rapid experiments an excessive amount was probably injected. The injections, for instance, were made at the rate of 4 cc. every 3 minutes. When the end-point was approached 1 cc. might have been sufficient to cause a toxic effect. Errors of this kind are smaller in the slower experiments because a smaller amount is given every 6 minutes.

Two experiments, Nos. 9 and 12 (Table I), illustrate these points. Four injections at different rates were given in each case. In No. 9 the first injection was given at the rate of 60 per cent in 15 minutes, and the toxic effect (minimum toxic dose) was produced with 44 per cent of the minimum lethal dose. 6 days later the same cat received an injection at the rate of 60 per cent in 2 hours and showed toxic manifestations with 47 per cent; the third injection, 6 days later, was given at the rate of 60 per cent in 1 hour and the minimum toxic dose was 35 per cent; the final one was given 4 days later at the rate of 60 per cent in 4 hours, and the minimum toxic dose was 43 per cent. Similar results were obtained in the four injections in No. 12. The rate at which the

injections were given was also 60 per cent of the minimum lethal dose in varying intervals of time. The variations were 48 per cent for 15 minutes, 36 per cent for 30 minutes, 42 per cent for 1 hour, and 36 per cent for 4 hours. In these instances, then, the minimum toxic dose is independent of the rate of injection. To test the point further, the rate of injection was changed in several instances during the same experiment. If a cat at first was given 30 per cent in 1 minute, injection was continued after waiting 15 minutes at the rate of 60 per cent in 2 hours. The toxic effect again was produced after a certain total amount had been given.

*Group 2.*—Further evidence on the relation of rate of injection to amount injected was obtained from a series in which large single injections were made in 1 or 2 minutes. It is, of course, impossible in a given animal to forecast the minimum toxic dose.

TABLE III.

| Per cent of minimum lethal dose given in 1–2 min. | 25 | 30 | 35 | 40 | 50 | 55 | 60 | 70 | 75 | 80 | 90 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Result. | 4 N.* | 4 N. | | 1 N. | 2 N. | | 1 N. | | | | | |
| | 1 T. | 1 T. | 1 T. | 1 T. | 1 T. | 1 T. | 1 T. | 2 T. | | 1 T. | 1 T. | 1 T. |
| | | | | | | | | | 1 F. | 1 F. | 1 F. | 2 F. |

* N. indicates negative result; T., toxic effect; F., fatal.

It was therefore necessary in a series of animals to obtain it by trial. The averages then served for comparison. There were twenty-nine cats. They received single injections of amounts varying from 25 to 100 per cent of the minimum lethal dose. There was, as was expected, great variation in the minimum toxic dose, for some cats showed effects from 25 per cent, while others required 60 per cent of the minimum lethal dose (Table III). Of five experiments in which 25 per cent was given only one was toxic. One cat in five was found to be intoxicated by injections of 30 per cent; one in two experiments in which 40 per cent was injected; one in three 50 per cent injections, and one in two 60 per cent injections. All injections over 60 per cent of the minimum lethal dose caused toxic effects and many were also fatal. There was also some variation in the minimum lethal dose, for 75 per cent

was fatal to one cat while 100 per cent was not to another. It is difficult to calculate an average toxic dose from such experiments, but in general these figures agree with those of Table II. If flooding the circulation suddenly with a large dose of strophanthin were more toxic than giving the same dose continuously over an hour or two, one would expect a greater percentage of toxic and lethal effects than were obtained. When 30 per cent, for instance, was given, only one of five showed an effect.

These results throw doubt on the conclusion that strophanthin produces its effects in accordance with the concentration of the drug to which the heart is exposed. For the differences in the speed of administration must alter its concentration in the blood stream, the blood volume probably remaining constant. So far, then, the toxic effect appears to be produced when a given total amount has been injected into the blood. An objection to this conclusion may, however, be offered; strophanthin, no matter at what speed administered, may be supposed to remain inert in the blood stream until a certain amount has been given, to provide a concentration sufficient for activity. When the required concentration is reached the heart is intoxicated. The objection is hardly valid, for there is evidence that even before the toxic effects appear strophanthin affects the heart. The therapeutic effects, for instance, of the drug on the heart are obtained by subtoxic doses.

Cohn, Fraser, and Jamieson (34) have shown that the heart muscle may be affected by digitalis before toxic symptoms are produced. Robinson and Wilson (35) show that to bring about T wave changes in the electrocardiogram of cats only 23.9 per cent of the lethal dose is necessary. Gros (36), using very dilute solutions of strophanthin on the perfused frog's heart, produced intermittent diastolic pauses without any other toxic results. These observations suggest that throughout the experiments the cats' hearts take up strophanthin, the toxic symptoms appearing when a certain total amount has been taken up.

*Group 3.*—Transfusion experiments were undertaken to obtain information on the amount of the drug actually present in the blood.

*Experiment 18.*—Cat A, weight 4,150 gm. was given 0.1 mg. of strophanthin at 11.05 and 0.1 mg. at 11.15 a.m. At 11.20 ventricular ectopic tachycardia appeared. At 12.50 p.m., 75 cc. of blood were taken from left carotid artery into

citrate solution. From a second cat, B, weight 4,250 gm., (the minimum toxic dose having previously been found to be 66 per cent of the minimum lethal dose), 70 cc. of blood were withdrawn at 12.50 from the left carotid artery. At the same time 70 cc. of strophanthinized blood from Cat A were transfused into the right saphenous vein of Cat B. At 1.10 the injection of strophanthin intravenously was started at the rate of 0.03 mg. every 6 minutes. After 0.12 mg. had been given, ventricular ectopic tachycardia was noticed. The minimum toxic dose equalled 28 per cent of the minimum lethal dose. Death occurred after 0.225 mg. had been given. This equalled 53 per cent of the minimum lethal dose.

The low minimum toxic dose and the low minimum lethal dose indicate that some strophanthin must have been contained in the blood of Cat A, for it took much less additional strophanthin to produce either a toxic or a fatal result in Cat B than we know would otherwise have been required. It is impossible to calculate how much of the drug the transfused blood of Cat A contained, but the result makes it reasonable to say that 1 hour and 45 minutes after the toxic effect had been produced the blood of Cat A contained at least a moderate amount of strophanthin.

*Experiment 19.*—Cat A, weight 4,400 gm., was given 0.1 mg. of strophanthin at 10.26 and 0.1 mg. at 10.36 a.m. At 10.43 a toxic effect occurred. At 11.55 an added 0.2 mg. was given intravenously. At 12.05 p.m. 100 cc. of blood were taken from the left carotid artery. Then from a second cat, B, weight 3,300 gm. (the minimum toxic dose for which having previously been found to be 67 per cent of the minimum lethal dose), 70 cc. of blood were removed at 12.40. At 12.42, 85 cc. of blood from Cat A were transfused into the left saphenous vein of Cat B. Then at 1.12, 0.026 mg. of strophanthin was given every 3 minutes. A toxic effect was produced with 0.147 mg., the equivalent of 45 per cent of the minimum lethal dose, and death occurred after 0.303 mg. had been injected. This equalled 92 per cent of the minimum lethal dose.

This experiment also indicates that a certain amount of strophanthin was carried over in the blood from Cat A, for the minimum toxic dose for Cat B when first tested was 67 per cent of the minimum lethal dose and after transfusion was only 45 per cent.

*Experiment 20.*—Cat A, weight 2,900 gm., was given on Mar. 31 at 10.35 a.m., 0.1 mg. of strophanthin; at 10.53, 0.05 mg.; at 10.57 a toxic effect was observed. At 11.40, 70 cc. of blood were taken from the left femoral artery. At 11.45, 75 cc. of normal saline solution were given. At 12 m. 25 cc. of citrated blood from Cat B were injected; at 12.37 p.m., 0.05 mg. of strophanthin was given; at 12.42, 0.05 mg., and at 12.47, 0.02 mg. The fatal dose was 0.27 mg.; *i.e.*, 93 per cent of the minimum lethal dose.

On Mar. 27, the minimum toxic dose of Cat B, weight 2,600 gm. was found to be 77 per cent of the minimum lethal dose. On Mar. 31, at 11.45, 50 cc. of blood were taken from the right carotid artery. At 11.52, 65 cc. of strophanthinized blood from Cat A were transfused into Cat B. At 1.17, 0.063 mg. and at

1.23 an additional 0.063 mg. of strophanthin were injected. At 1.45 a toxic effect was produced. In this experiment, therefore, a toxic effect was produced with 48 per cent of the minimum lethal dose. At 1.48, 0.031 mg., and at 1.51, 0.031 mg. of strophanthin were added. The cat died at 1.57 after taking 72 per cent of the minimum lethal dose.

| Cat A, weight 2,900 gm. | | Cat B, weight 2,600 gm. (minimum toxic dose previously found to be 77 per cent of minimum lethal dose). | |
|---|---|---|---|
| *a.m.–p.m.* | | *a.m.–p.m.* | |
| 10.35 | 0.1 mg. of strophanthin. | | |
| 10.53 | 0.05 " " " | | |
| 10.57 | Toxic effect. | | |
| 11.40 | Bled 70 cc. | | |
| 11.45 | 75 cc. of saline solution intravenously. | 11.45 | Bled 50 cc. |
| | | 11.52 | 65 cc. of strophanthinized blood of Cat A. |
| 12.00 | 25 cc. of blood from Cat B. | | |
| 12.37 | 0.05 mg. of strophanthin. | | |
| 12.42 | 0.05 " " " | | |
| 12.47 | 0.02 " " " | | |
| 12.51 | Died. | | |
| | | 1.17 | 0.063 mg. of strophanthin. |
| | | 1.23 | 0.063 " " " |
| | | 1.45 | Toxic effect. |
| | | 1.48 | 0.031 mg. of strophanthin. |
| | | 1.51 | 0.031 " " " |
| | | 1.57 | Died. |

Minimum toxic dose for Cat A = 52 per cent of the minimum lethal dose. Lethal dose for Cat A = 93 per cent of the minimum lethal dose. Minimum toxic dose for Cat B = 65 cc. of strophanthinized blood of Cat A plus 48 per cent of the minimum lethal dose. Minimum lethal dose for Cat B = 65 cc. of strophanthinized blood of Cat A plus 72 per cent of the minimum lethal dose.

From Cat A, then, 43 minutes after the toxic effect was produced about half[4] of the blood was removed (70 cc.). Some strophanthin must have been removed with the blood; however, 93 per cent of the minimum lethal dose was required to cause death. Cat B, which required 77 per cent of the minimum lethal dose to produce toxic effects in the preliminary test, was toxic after the injection of the 65 cc. of blood taken from Cat A, plus 48 per cent of the minimum lethal dose. It died after 72 per cent was injected. This amount was less than the minimum toxic dose on the first occasion.

[4] Calculated on the basis that 5.5 per cent of cat's weight is blood. (Ellenberger, W., and Scheunert, A., Lehrbuch der vergleichenden Physiologie der Haussäugetiere, Berlin, 1910.)

*Experiment 21.*—The minimum toxic dose of Cat A, weight 3,850 gm., was found to be 72 per cent of the minimum lethal dose. On Apr. 3, at 11 a.m., 0.092 mg. of strophanthin was injected, and at 11.15 an additional 0.08 mg. (47 per cent). No toxic effect was produced. At 12.42 p.m., 90 cc. of blood were withdrawn from the left femoral artery. At 12.46, 45 cc. of blood taken from a normal cat were transfused. At 12.55, 25 cc. of normal saline solution were given. Beginning at 1.06, 0.046 mg. of strophanthin was given every 6 minutes until death. The toxic effect occurred after an additional 34 per cent of the minimum lethal dose (total 81 per cent) and the lethal dose after 116 per cent was injected.

This experiment shows in a somewhat different manner from the previous transfusion experiments that an appreciable amount of strophanthin must have been removed in bleeding, for although 47 per cent of the minimum lethal dose was given without producing a toxic effect, 34 per cent more had to be given after the bleeding to cause the toxic symptoms. If all the strophanthin had been bound either to the heart or to the other tissues so much would hardly have been required. The fatal dose was in excess of the expected amount if all had been retained in the body. Of the four transfusion experiments No. 21 was the only one which showed a larger minimum toxic dose than was previously found in the corresponding cat, and it was also the only one in which the minimum lethal dose was more than 100 per cent.

These results are in accord with the idea that strophanthin remains in the blood stream, for while in Experiments 18 to 20, strophanthinized blood from other cats was added, in Experiment 21 strophanthinized blood was removed. These transfusion experiments are imperfect and do not permit an accurate calculation of the concentration of the drug in the blood stream. They show, however, that an appreciable amount is present in the peripheral circulation an hour or more after intravenous administration, whether a toxic effect is produced or not.

Strophanthin almost invariably caused death by fibrillation of the ventricles; respiration continued longer. There is no way of preventing this or of stopping it after its inception. If a fatal issue depends on the presence of strophanthin in the blood, the withdrawal of blood should prevent or delay death. An attempt was accordingly made after injecting 100 per cent of the minimum lethal dose to prevent ventricular fibrillation by bleeding the animals as soon as the toxic effect was observed. This procedure failed in three experiments. Whether a fatal outcome would

occur if the drug were given slowly was not ascertained. Ether never caused fibrillation of the ventricles; respiration stopped, but the electrocardiogram showed the presence of heart block for a long time. Massaging the heart when the ventricles fibrillate, frequently delayed death for ½ hour. The pupils remained small, and respiration continued. If massage was stopped the pupils dilated and respirations became labored or ceased; on resuming massage the pupils again became small, and the respirations more easy. In the absence of any other treatment massage of the heart offers a possible method of treating fibrillation, although the chances are against a favorable outcome. In all but one instance the cat finally died.

The outcome of this exceptional case, Cat 7 b, is important, for no other case of recovery from digitalis poisoning in an experimental animal has been recorded. This cat was given 0.013 mg. of strophanthin every 6 minutes; the ether cone was kept in place but no ether was added after the injections began. In 49 minutes, when 52 per cent of the minimum lethal dose had been given, extrasystoles were observed (Fig. 4); the injections were stopped, but 10 minutes later the ventricles fibrillated. Electrocardiograms were obtained at frequent intervals during this time. The heart was massaged without opening the chest. For a time the galvanometer string was practically motionless, then unusual deflections were seen, and later a return to typical ventricular fibrillation; finally ventricular tachycardia developed. The breathing improved; the pupils, which were at times markedly dilated, became small, and the cat recovered. The last tracing (Fig. 4), taken 22 minutes after the onset of ventricular fibrillation, shows a normal cardiac mechanism except for delayed conduction (P–R) time, 0.16 second. 6 days later the animal was used again for an experiment.

A number of details deserve notice. (1) In one experiment (No. 20) ventricular ectopic tachycardia did not appear until 90 per cent of the minimum lethal dose had been injected. The warning is therefore expressed that intoxication may be present, without the appearance of extrasystoles.

(2) In fractional administration irregularities usually occurred ½ to 4 minutes after the last injection. Their duration was usually longer than 30 minutes. Durations of 30 and 50 minutes were observed in two cats, and in seven other cats, of ½ to 4 hours. After single large doses, irregularities began in 2 to 15 minutes. Death occurred in 9 to 30 minutes.

(3) Numerous fatalities in patients have resulted from the intravenous administration of strophanthin, but most of them have occurred when this drug was given to patients who recently had taken digitalis, or when large doses were repeated on the same day. It is

TABLE IV.

| Experiment No. | Method of administration. | Minimum toxic dose. | Minimum lethal dose. | Margin of safety. | |
|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | |
| 3 b | 40 per cent of minimum lethal dose in 2 hrs. | 47 | 70 | 23 | (23)* |
| 22 c | 40 per cent in 1 min. was toxic; 2½ hrs. later irregularity persisted and 20 per cent was fatal. | 40 | 60 | 20 | (25) |
| 5 c | 40 per cent of minimum lethal dose in 2 hrs. | 40 | 78 | 38 | (43) |
| 6 c | 50 per cent of minimum lethal dose in 2 hrs. | 30 | 45 | 15 | (19) |
| 7 c | 52 per cent in 1 min. was toxic; 1 hr., 20 min. later irregularity had disappeared and 20 per cent in 1 min. was fatal. | 52 | 72 | 20 | (27) |
| 8 c | 60 per cent in 2 hrs. | 40 | 55 | 15 | (22) |
| 23 a | 70 per cent in 2 min. was toxic; 1 hr., 50 min. later heart again regular and 30 per cent in 1 min. was fatal. | 70 | 100 | 30 | (30) |
| 10 b | 60 per cent in ½ hr. | 71 | 89 | 18 | (20) |
| 12 d | 60 per cent in 4 hrs.; waited 1 hr. after toxic effect; heart irregular then; 6 per cent in 1 min. was fatal. | 36 | 42 | 6 | ( 8) |
| 20 a | 60 per cent in ½ hr. | 54 | 96 | 42 | (42) |
| 21 a | 60 " " " ½ " | 72 | 90 | 18 | (18) |

* All percentages were calculated on the basis of the original weight of the animal. The figures in parentheses are calculated on the actual weight and are slightly higher.

impossible from these experiments to make recommendations for the administration of strophanthin to man. They show that a margin of safety exists, represented by the difference between the minimum lethal dose and the minimum toxic dose. This difference (Table IV)

varied between 6 and 42 per cent of the minimum lethal dose. To predict the toxic dose is, therefore, impossible. The dose, for example, that was fatal to Cat 12 was not even toxic for Nos. 3, 10, 20, and 21. To foretell the toxic dose in patients is probably equally difficult. But since these experiments show that the same results can be obtained from slow as from rapid injections, a fractional method of administration suggests itself. If, for instance, 0.5 mg. given in a single injection were fatal, 0.2 mg. might be given followed by 0.1 mg. repeated every half hour until 0.5 mg. is given. It may be possible to regulate the procedure by watching for signs of intoxication, either by auscultating for irregularities or by taking graphic records, to notice changes in the T waves or the P–R time. Under these conditions, giving an excess of more than 0.1 mg. is avoided. Treatment should not be repeated within 24 hours. Vaquez and Lutembacher (37) have reported almost 2,000 intravenous injections of ouabain without harm or fatality.

## DISCUSSION AND SUMMARY.

The review of the literature relating to experiments on the action of the digitalis glucosides shows that care must be exercised in estimating the significance of the results published. The perfusion of isolated hearts obviously cannot take into account the question of excretion of the drug or its combination with other tissues. Nor can perfusion experiments take into account the influence of the extracardiac nerves. Vagotomy alone, as Macht and Colson (38) have shown, has a marked influence on the result. And Richards and Wood (39) have shown that the effect of the intravenous injection of strophanthin is altered after section of the splanchnic nerves. Another difficulty in making deductions of a general nature lies in the fact that digitalis bodies differ in their action, in their solubility, and in the rate of their absorption. These difficulties are made clear when the following statements are placed side by side. Strophanthin is much more toxic to a frog's heart than digitonin, but the latter is more toxic to the isolated sartorius (40). Voegtlin and Macht (41), experimenting on isolated coronary arteries, found that digitonin and digalen produced relaxation of the

arteries, while digitoxin and digitalin caused constriction. Fraser (42), many years ago, called attention to the fact that while strophanthin was much more profound in its action on the frog's heart than digitalin, the reverse was true as to their action on the blood vessels.

There is also a marked difference in the reaction and susceptibility of different animals to these drugs. And the problem is further complicated by the change in the mode of administration from animal to animal. The intact grass snake, for instance, is 30 times more tolerant to strophanthin given subcutaneously than is the frog, but the isolated heart is 1,000 times as tolerant. The action of these drugs depends upon the state of the heart, on the temperature of the surrounding media, the proportion of inorganic salts in the fluid, the pressure to which the heart is exposed, the rate of the heart beat, the presence or absence of blood corpuscles or serum, and, no doubt, other factors as well. To obtain identical results under artificial conditions means that all these factors must be controlled. These are requirements which are obviously difficult to meet. The experiments now reported have avoided these difficulties.

The experiments have a bearing on the controversy between Straub and Grünwald. Straub believes that the action of strophanthin is independent of the total amount and depends entirely upon the concentration; Grünwald holds that although the concentration is the prime factor when large quantities of the active principle are used, the total amount is of importance when the dilutions are small and when small amounts are compared. The distinction is important because it is the small amounts and the low dilutions that are employed in clinical medicine. Furthermore, it is not safe to measure the toxicity of digitalis in terms of the time it takes to produce standstill of the ventricles, since Schmiedeberg (43) found that the two factors did not vary directly.

In these experiments, in which crystalline strophanthin was injected intravenously, the amount of the drug needed to produce toxic results as shown electrocardiographically was practically independent of the speed of administration. The use of the same animal for repeated experimentation avoided the error which re-

sults from making comparisons between different species of animals, and eliminated the marked variations due to individual susceptibility. Injections of the drug were made at varying speeds, with intervals of 4 or more days between experiments. The total amount required to produce the toxic effect did not vary significantly. The experiments (Table II) show strikingly that the total amount required to produce a toxic effect is independent of the speed of administration. This conclusion is probable because although the amount was the same, the concentration was considerably greater when 60 per cent of the minimum lethal dose was given in a single dose or in $\frac{1}{4}$ hour than when given in 4 hours. It has been suggested that when the drug is given slowly, it may remain inert in the blood stream and produce a toxic effect only when a proper concentration is reached, this concentration being reached when the required amount has been injected without reference to the speed of administration. Against this view we have noted the fact that the heart is known to be affected by the drug before toxic effects are produced. The transfusion experiments were undertaken to determine whether strophanthin was present in the blood stream. The method showed that strophanthin must be regarded as present and capable of remaining there for a moderate time but proved too crude to give an idea of its actual concentration. A theory of the action of strophanthin may be formulated from the above considerations. It reconciles the conflicting views relating to the importance of concentration and of total amount of the drug. It supposes that the time required to produce a given effect in a heart varies inversely with the concentration of the active principle. The heart utilizes only a small portion (in the neighborhood of 10 per cent) of the drug to which it is exposed no matter what the concentration. A toxic effect results when the heart has taken up a certain total amount of the drug which is a definite small fraction of its own weight. If this theory is correct, it explains why in concentrated solutions the total amount is not important, for the small part that is taken out by the heart does not appreciably alter the concentration, while when very dilute solutions or small quantities are used the amount taken up by the heart diminishes the remaining concentration appreciably;

that is, the "digitalis pressure" becomes lessened. In these experiments the rapid injections forced an adequate amount of strophanthin into the heart rapidly and produced the toxic effect; in the slow injections the same total amount of drug was taken up by the heart only more slowly.

Experiments were carried out on a series of cats in which, after the minimum toxic dose was determined, the injections (some were slow, others more rapid) were continued until death. In this way the margin of safety was determined, the difference between the minimum lethal dose and the minimum toxic dose. It varied from 6 to 42 per cent of the minimum lethal dose.

These experiments have an importance in clinical medicine. They suggest a method for intravenous medication with strophanthin, designed to reduce the danger due to using the drug. After the ventricles have fibrillated, there is no constant way of reviving a heart. Occasionally, as in one experiment, resuscitation may take place after ventricular fibrillation and standstill, by massage of the heart without opening the chest. No opinion can be offered on the practical value of this method.

## CONCLUSIONS.

1. Cats vary considerably in their susceptibility to strophanthin and in the extent of the difference between the minimum lethal dose and the minimum toxic dose.

2. The amount of strophanthin necessary to produce a toxic effect in a given cat is independent of the speed of administration to a period of 4 hours. An improvement in the clinical administration of the drug is thereby indicated.

3. A theory of the action of strophanthin is formulated which reconciles the results which point to the importance of the total amount taken up by the heart with those which indicate that the concentration of the drug is the determining factor.

## BIBLIOGRAPHY.

1. Straub, W., *Biochem. Z.*, 1910, xxviii, 392.
2. Holste, A., *Arch. exp. Path. u. Pharmakol.*, 1912, lxx, 435.
3. Grünwald, H. F., *Arch. exp. Path. u. Pharmakol.*, 1912, lxviii, 231.

4. Straub, W., *Arch. exp. Path. u. Pharmakol.*, 1913, lxxi, 139.
5. Weizsäcker, V., *Arch. exp. Path. u. Pharmakol.*, 1913, lxxii, 347.
6. Clark, A. J., *Proc. Roy. Soc. Med.*, 1911–12, v, Therap. and pharmacol. sect., 181.
7. Issekutz, *Arch. exp. Path. u. Pharmakol.*, 1915, lxxviii, 155.
8. Trendelenburg, P., *Arch. exp. Path. u. Pharmakol.*, 1909, lxi, 256.
9. Gunn, J. W. C., *J. Pharmacol. and Exp. Therap.*, 1914–15, vi, 39.
10. Sollmann, T., Mendenhall, W. L., and Stingel, J. L., *J. Pharmacol. and Exp. Therap.*, 1914–15, vi, 533.
11. Weizsäcker, V., *Arch. exp. Path. u. Pharmakol.*, 1913, lxxii, 282.
12. Clark, A. J., *J. Pharmacol. and Exp. Therap.*, 1913–14, v, 215.
13. Pittenger, P. S., and Vanderkleed, C. E., *J. Am. Pharm. Assn.*, 1915, iv, 427.
14. Williams, F., *Arch. exp. Path. u. Pharmakol.*, 1881, xiii, 1.
15. Krailsheimer, R., *Arch. exp. Path. u. Pharmakol.*, 1910, lxii, 296.
16. Jacobj, *Arch. exp. Path. u. Pharmakol.*, 1900, xliv, 368.
17. Wybauw, R., *Arch. exp. Path. u. Pharmakol.*, 1900, xliv, 434.
18. Cushny, A. R., and Gunn, J. A., *J. Pharmacol. and Exp. Therap.*, 1913–14, v, 1.
19. Gröber, A., *Arch. exp. Path. u. Pharmakol.*, 1913, lxxii, 317.
20. Fraser, T. R., *Tr. Roy. Soc. Edinburgh*, 1890, xxxv, 955.
21. Werschinin, N., *Arch. exp. Path. u. Pharmakol.*, 1909, lx, 328.
22. Clark, A. J., *J. Pharmacol. and Exp. Therap.*, 1912–13, iv, 399.
23. Burridge, W., *Quart. J. Med.*, 1916, ix, 271.
24. Cloetta, M., and Fischer, H. F., *Arch. exp. Path. u. Pharmakol.*, 1906, liv, 294.
25. Hatcher, R. A., *J. Am. Med. Assn.*, 1913, lxi, 386.
26. Hatcher, R. A., *Am. J. Physiol.*, 1908–09, xxiii, 303.
27. Hatcher, R. A., and Bailey, H. C., *J. Am. Med. Assn.*, 1909, lii, 5.
28. Schliomensun, B., *Arch. exp. Path. u. Pharmakol.*, 1910, lxiii, 294.
29. Halsey, J. T., *J. Exp. Med.*, 1917, xxv, 729.
30. Cohn, A. E., *Heart*, 1913–14, v, 5.
31. Christian, H. A., *Arch. Int. Med.*, 1915, xvi, 341.
32. Lewis, T., White, P. D., and Meakins, J., *Heart*, 1913–14, v, 289.
33. Hatcher, R. A., *Arch. Int. Med.*, 1912, x, 268.
34. Cohn, A. E., Fraser, F. R., and Jamieson, R. A., *J. Exp. Med.*, 1915, xxi, 593.
35. Robinson, G. C., and Wilson, F. N., *J. Pharmacol. and Exp. Therap.*, 1917–18, x, 491.
36. Gros, O., *Arch. exp. Path. u. Pharmakol.*, 1913, lxxi, 364.
37. Vaquez and Lutembacher, *Bull. Acad. méd.*, 1917, lxxvii, 405.
38. Macht, D. I., and Colson, H., *J. Pharmacol. and Exp. Therap.*, 1917, ix, 343.
39. Richards, A. N., and Wood, W. G., *J. Pharmacol. and Exp. Therap.*, 1914–15, vi, 283.
40. Clark, A. J., *Brit. Med. J.*, 1912, ii, 687.

41. Voegtlin, C., and Macht, D. I., *J. Pharmacol. and Exp. Therap.*, 1913–14, v, 77.
42. Fraser, T. R., *Tr. Roy. Soc. Edinburgh*, 1891, xxxvi, 343.
43. Schmiedeberg, O., *Arch. exp. Path. u. Pharmakol.*, 1910, lxii, 305.

## EXPLANATION OF PLATES.

### PLATE 23.

FIG. 1, *a*. Cat 12 d. This electrocardiogram was taken at 2.40 p.m. before strophanthin injections.

*b*. This curve was taken at 4.44 when 36 per cent of the minimum lethal dose had been given. It shows ventricular extrasystolic tachycardia, the typical toxic effect.

*c*. Curve taken at 5.43. 10 per cent in addition had been given at 5.42 (total of 46 per cent of the minimum lethal dose). It shows typical ventricular fibrillation.

FIG. 2, *a*. Cat 6 b. Curve taken at 3.35 p.m. before strophanthin was injected. It shows normal heart mechanism.

*b*. Curve taken at 4.13 after 35 per cent of the minimum lethal dose had been given. It shows a toxic effect in which the ventricular complex is continually changing. There are alterations also in the P–R relation.

### PLATE 24.

FIG. 3, *a*. Cat 12 a. Curve taken at 4.57 p.m. before strophanthin injection. The first five contractions are nodal beats.

*b*. Curve taken at 5.05 after 20 per cent of the minimum lethal dose of strophanthin had been given. It shows a nodal rhythm; the auricular waves are lost in the ventricular complexes.

*c*. Curve taken at 5.07 after 28 per cent of the minimum lethal dose. It shows that the heart has returned to the normal mechanism.

### PLATE 25.

FIG. 4, *a*. Cat 7 b. Curve taken at 12.15 p.m., before strophanthin was given. It shows normal rhythm.

*b*. Curve taken at 1.06 p.m., after 52 per cent of the minimum lethal dose had been given. It shows frequently changing ventricular complexes.

*c*. Curve taken at 1.15. No additional strophanthin. The curve shows ventricular fibrillation in the first portion and then a standstill of the heart.

*d*. Curve taken at 1.30 showing the gradual onset of a ventricular extrasystolic tachycardia.

*e*. Curve taken at 1.37 showing sequential contraction of auricles and ventricles but with delayed conduction; P−R = 0.16 second.

# A FUNCTIONAL AND PATHOLOGICAL STUDY OF THE CHRONIC NEPHROPATHY INDUCED IN THE DOG BY URANIUM NITRATE.*

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Plates 26 to 30.

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Since the initial observation in 1888 by Chittenden and Hutchinson[1] that uranium salts would induce an acute nephropathy, these substances have been extensively used as acute nephrotoxic agents. Only a few observations have been made of the effect of these salts on the kidney in prolonged intoxications. Such observations as have been made are largely concerned with the type of the pathological response on the part of the kidney, and a study of the processes of repair which take place in the kidney during its recovery from the acute injury.

In 1904 Richter[2] employed uranium to induce a condition in animals analogous to Bright's disease. As a result of Richter's observations, uranium was employed by Dickson[3,4] in two researches, in which he demonstrated that prolonged intoxications by uranium nitrate would induce in guinea pigs, rabbits, and dogs a kidney injury which was comparable with some of the types of chronic kidney disease found in man. The form of injury most frequently induced was

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[1] Chittenden, R. H., and Hutchinson, M. T., Some experiments on the physiological action of uranium salts, *Tr. Conn. Acad. Arts and Sc.*, 1888–92, viii, 1.

[2] Richter, P. F., Die experimentelle Erzeugung von Hydrops bei Nephritis, *Beitr. klin. Med.*, Festschrift, Professor Senator, Berlin, 1904, 283.

[3] Dickson, E. C., A report on the experimental production of chronic nephritis in animals by the use of uranium nitrate, *Arch. Int. Med.*, 1909, iii, 375.

[4] Dickson, E. C., A further report on the production of experimental chronic nephritis in animals by the administration of uranium nitrate, *Arch. Int. Med.*, 1912, ix, 557.

of a diffuse interstitial type which in some instances went to the stage of granular atrophy. Associated with these later stages, certain of the animals became polyuric.

The present investigation has been undertaken with the object in view of studying the functional capacity of the kidney during the period of acute injury from uranium, and also during the period when the kidney is recovering from the acute degeneration and passing into that stage of chronic injury which is characterized by such changes in structure that the kidneys may be considered to represent some type of chronic nephropathy. The investigation has a further object. In previous papers[5,6] the observation has been made that the acute injury to the normal kidney, or to the naturally nephropathic kidney, by uranium is associated with the development of an acid intoxication. In the present study observations will be made of the changes in the acid-base equilibrium of the blood of these animals, not only during the period of acute damage to the kidney, but during the period of recovery from such an injury, when the relation may be studied of such changes in the blood with the processes of repair in the kidney, and the return of the functional response of the kidney.

## EXPERIMENTAL.

Twenty-seven female dogs have been used in this series of experiments. The animals have varied from 5 months to $10\frac{1}{2}$ years in age. They were kept in metabolism cages for 4 days prior to the commencement of the intoxication. During this period studies of the urine, blood, and the functional capacity of the kidney were made, in order to eliminate animals with a naturally acquired nephropathy. All the animals were free from renal disease. The animals were given 500 cc. of water daily, and fed on bread with

[5] MacNider, W. deB., The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, *J. Exp. Med.*, 1916, xxiii, 171.

[6] MacNider, W. deB., The stability of the acid-base equilibrium of the blood in naturally nephropathic animals, and the effect on renal function of changes in this equilibrium. I, *J. Exp. Med.*, 1918, xxviii, 501.

a small amount of cooked lean beef. The animals were catheterized once a day, and the amount of urine obtained was added to the cage urine for analysis. The experiments were terminated at various periods during the intoxication without employing an anesthetic. This method of termination eliminates the probability of inducing acute degenerative changes in the liver as well as the kidney. The animals were poisoned by one dose of 4 mg. of uranium nitrate per kilo. The uranium was given subcutaneously.

During the course of the experiments the urine was examined quantitatively for albumin by Esbach's method, and for glucose with Benedict's reagent. The functional capacity of the kidney was studied by noting the percentage retention of blood urea, as shown by Marshall's[7] method, as modified by Van Slyke and Cullen,[8] and also by the elimination of phenolsulfonephthalein. The latter functional test was conducted according to the technique of Rowntree and Geraghty.[9] Observations on the acid-base equilibrium of the blood were made by employing the methods of Marriott[10,11] by ascertaining the alkali reserve of the blood and the tension of alveolar air carbon dioxide.

*The Course of Prolonged Intoxication by Uranium in the Dog.*

A large number of preliminary experiments has been necessary, in order to determine the dose of uranium which would effect a severe renal injury, and yet not be sufficiently toxic to injure the kidney so that processes of repair could not arise and lead to the development of a chronic nephropathy. As a result of these pre-

[7] Marshall, E. K., Jr., A rapid clinical method for the estimation of urea in urine, *J. Biol. Chem.*, 1913, xiv, 283.

[8] Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease, and its use in the determination of urea, *J. Biol. Chem.*, 1914, xix, 211.

[9] Rowntree, L. G., and Geraghty, J. T., An experimental and clinical study of the functional activity of the kidneys by means of phenolsulphonephthalein, *J. Pharmacol. and Exp. Therap.*, 1909–10, i, 579.

[10] Marriott, W. McK., A method for the determination of the alkali reserve of the blood plasma, *Arch. Int. Med.*, 1916, xvii, 840.

[11] Marriott, W. McK., The determination of alveolar carbon dioxide tension by a simple method, *J. Am. Med. Assn.*, 1916, lxvi, 1594.

liminary experiments, an observation previously made by me[12] was confirmed, that the toxicity of uranium, as expressed by the severity of the kidney injury, largely depended upon the age of the animal.

A study of the experiments which follow not only illustrates the relative toxicity of uranium for animals of different ages, but also shows that young animals may so repair the damage done the kidney by uranium that they can, with the development of a chronic type of nephropathy, reestablish to some extent the function of the kidney, while in the older animals these changes of repair do not occur.

As a result of these observations it has seemed advisable to group the experiments used in this study according to the age of the animal. Group 1 is represented by seven animals between 6 and $10\frac{1}{2}$ years of age; Group 2, by eight animals varying from 1 to 4 years of age; while Group 3 includes twelve animals from 5 to 11 months of age. From the experiments in the different groups of animals, the course of the intoxication, as illustrated by one experiment typical for the group, is included in the form of a table. In order to reduce the space required for the tables, only observations on every 3rd day of the experiment have been tabulated.

*Group 1. Animals Varying in Age from 6 to $10\frac{1}{2}$ Years.*

Seven animals are included in this group. Four of the experiments were artificially terminated, one on the 6th, 8th, 10th, and 12th days respectively following the commencement of the uranium intoxication. Of the remaining three animals of the group two died on the 10th day of the intoxication in a comatose condition, which rapidly developed following a period of convulsions. The third animal died on the 6th day in air-hunger. None of the animals of this group survived the intoxication for longer than 12 days.

Reference to Table I shows the course of the intoxication in an animal representative of this group. During the first 24 hour period following the injection of uranium there occurs an increase

[12] MacNider, W. de B., A consideration of the relative toxicity of uranium nitrate for animals of different ages. I, *J. Exp. Med.*, 1917, xxvi, 1.

in the output of urine. At this early period both albumin and glucose appear in the urine, and the centrifugalized urine shows both hyaline and granular casts. Evidence of kidney injury is indicated by the sudden reduction in the elimination of phenolsulfonephthalein and by a retention of blood urea. In Experiment 1 the output of phenolsulfonephthalein was reduced within 24 hours from the normal elimination of 58 per cent to 20 per cent, while the blood urea showed a retention of 0.028 per cent as opposed to the normal of 0.015 per cent.

The acid-base equilibrium of the blood in this group of old animals has shown a similar abrupt change in favor of a decrease in the alkali reserve. The reserve alkali in Experiment 1 was re-

TABLE I.

*Prolonged Uranium Intoxication. Group 1, Experiment 1.*

| Day of experiment. | Age and weight of animal. | Water in 24 hrs. | Uranium nitrate per kg. | Urine in 24 hrs. | Albumin. | Glucose. | Blood urea. | Phthalein. | R. pH. | Carbon dioxide tension. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *cc.* | *mg.* | *cc.* | *gm.* | *per cent* | *per cent* | *per cent* | | *mm.* |
| 1 | 7 yrs. 12.35 kg. | 500 | 0 | 471 | 0 | 0 | 0.015 | 58 | 8.1 | 41 |
| 2 | | 500 | 4 | 760 | 1.3 | 0.9 | 0.028 | 20 | 8.0 | 37 |
| 5 | | 500 | 0 | 419 | 4.0 | 1.75 | 0.040 | Tr. | 7.8 | 29 |
| 8 | | 500 | 0 | 58 | 3.6 | 0.97 | 0.078 | 0 | 7.75 | 21 |

duced from 8.1 to 8, while the tension of alveolar air carbon dioxide was reduced from 41 mm. to 37 mm.

The progress of the intoxication in this group of old animals, as shown by Table I, has consisted in a rapid reduction in the formation of urine, an increase in the amount of both albumin and glucose in the urine, and a progressive decrease in the functional capacity of the kidney, as shown by the elimination of phenolsulfonephthalein and the retention of blood urea. This functional change in the kidney is associated with a rapid decrease in the alkali reserve of the blood and an associated reduction in the tension of carbon dioxide in alveolar air. Both the functional response of the kidney and the reduction in the alkali reserve of the blood are progressive in severity with the duration of the experiment. In

this group of old animals there has been no attempt at a restoration of renal function and no reestablishment of a normal acid-base equilibrium.

The kidneys from the animals of this group have shown no characteristic gross pathological change. The surface is smooth and the capsule has not been adherent. On section the cortex is pale. The tissue at the corticomedullary junction has shown a large amount of fat, which extends into the cortex in streaks. The histological study has shown a degree of degeneration which depends for its severity and extent upon the duration of the intoxication. In the animals of this group in which the kidneys were obtained prior to the 8th day, the degenerative changes have been largely confined to the tubules. These changes have consisted in an acute swelling and vacuolation, which first appears in the third portion of the convoluted tubule. This change is followed by pyknosis of the nuclei and necrosis of the epithelium. Even at this early stage of the intoxication the ascending limb of Henle's loops contains a large amount of fat. The accumulation of fat is more marked in the kidneys of this older group of animals than in any of the remaining younger groups.

By the 10th to the 12th days of the intoxication the epithelial injury has become more diffuse. All the proximal convoluted tubule has participated in the degeneration, and the epithelium of the distal convoluted tubules and junctional tubules has shown evidence of injury. In addition to the extension of the epithelial injury the vascular tissue of the kidney becomes involved in the degeneration. The intertubular and glomerular capillary endothelium has shown a peculiar hyaline type of change, and later becomes vacuolated. Following this degeneration of the vascular tissue exudates occur between the tubules and into the capsular space of the glomeruli (Fig. 1).

In this group of old animals there has been no indication of an attempt to repair the injury. There is no evidence of nuclear division.

*Group 2. Animals Varying in Age from 1 to 4 Years.*

Eight animals are included in this group. Three of the experiments were terminated on the 6th day and two on the 10th day. Of the remaining animals, one died on the 14th day and two on the 21st day.

The course of the intoxication in the animals of this age grouping resembles in general the response obtained with the older animals of Group 1.

The injection of the animals of this group with 4 mg. of uranium nitrate is followed within 24 hours by the appearance of both albumin and glucose in the urine (Table II). Following an initial increase in the formation of urine the daily output is rapidly reduced so that by

TABLE II.

*Prolonged Uranium Intoxication. Group 2, Experiment 2.*

| Day of experiment. | Age and weight of animal. | Water in 24 hrs. | Uranium nitrate per kg. | Urine in 24 hrs. | Albumin. | Glucose. | Blood urea. | Phthalein. | R. pH. | Carbon dioxide tension. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *cc.* | *mg.* | *cc.* | *gm.* | *per cent* | *per cent* | *per cent* | | *mm.* |
| 1 | 3 yrs. 14.7 kg. | 500 | 0 | 400 | 0 | 0 | 0.015 | 68 | 8.0 | 42 |
| 2 | | 500 | 4 | 413 | 0.5 | 0.61 | 0.015 | 63 | 7.95 | 37 |
| 5 | | 500 | 0 | 634 | 0.7 | 1.25 | 0.018 | 5 | 7.8 | 27 |
| 8 | | 500 | 0 | 450 | 1.3 | 1.87 | 0.023 | Tr. | 7.8 | 25 |
| 11 | | 500 | 0 | 210 | 0.9 | 1.03 | 0.031 | " | 7.75 | 21 |
| 14 | | 500 | 0 | 35 | 0.5 | 0.87 | 0.080 | 0 | 7.65 | 18 |

the 14th day, as indicated in Experiment 2, only 35 cc. of urine were formed. Within a few hours of the appearance of albumin in the urine there is a reduction in the reserve alkali of the blood, a decrease in the elimination of phenolsulfonephthalein, and a retention of blood urea. These changes in the functional capacity of the kidney and in the acid-base equilibrium of the blood progress in severity with the duration of the experiment.

A study of tables of animals representative of Groups 1 and 2 (Tables I and II) shows the following variations in the response of the animals of the two groups to the intoxication. The older animals of Group 1 show a more rapidly developing intoxication, as indicated by an earlier reduction in the formation of urine, by the urine's contain-

ing a larger amount of both albumin and glucose, and by the more rapid development of a severe kidney injury. In addition to these variations in the severity of the intoxication in the two groups of animals, a similar variation has occurred in the rate with which the alkali reserve of the blood in the two groups shows a depletion. By the 8th day of the intoxication in Experiment 1 of Group 1 the alkali reserve had been reduced to 7.75, while in Experiment 2 of the younger group of animals, Group 2, this degree in the reduction of the alkali reserve was obtained on the 11th day.

A study of the rate with which the kidney shows evidence of injury in the two groups indicates that the kidneys of the older animals are more susceptible to injury than is the case with the younger group of animals. In Experiment 1 of Group 1, within the first 24 hour period the elimination of phenolsulfonephthalein was reduced from 58 per cent to 20 per cent, and a retention of blood urea developed of 0.028 per cent as compared with the normal of 0.015 per cent.

In the younger animals of Group 2, as illustrated by Experiment 2, such a rapid injury to the kidney did not develop. During the first 24 hour period of the intoxication the elimination of phenolsulfonephthalein was only reduced from 68 per cent to 63 per cent, and during this same period there was no retention over the normal in blood urea.

The pathological study of the kidneys from the animals of Group 2 has shown only one outstanding difference from the pathological reaction described as occurring in the animals of Group 1. In both groups the epithelium of the convoluted tubules has shown the most marked evidence of injury. The type of injury has been the same. The kidneys of the younger group of animals, Group 2, that have been obtained after the 6th day of the intoxication, show definite evidence of repair by a beginning regeneration of tubular epithelium (Fig. 2). The regeneration first appears in the third or terminal portion of the convoluted tubule. In the cells of this portion of the tubule which have sufficiently withstood the process of degeneration, new epithelium is formed by a process of indirect cell division. At first the newly formed cells are large and have a clear cytoplasm. Later these cells flatten out and contain a small amount of deeply staining cytoplasm in proportion to the large hypochromatic nuclei.

In Fig. 2 the different stages in the regeneration of atypical cells in the convoluted tubules is clearly shown.

In this second group of animals which differ from the first group in that an attempt at repair has commenced in the kidneys, the processes of repair have not sufficiently progressed to be expressed by any improvement in the functional capacity of the kidney or by any change in the direction of restoring the normal acid-base equilibrium of the blood.

*Group 3. Animals Varying in Age from 5 to 11 Months.*

Twelve animals are included in this group. Two of the experiments were terminated on the 6th day of the intoxication, four on the 16th day, two on the 20th, two on the 35th day, and the remaining two experiments on the 48th day.

A study of Tables III and IV, of Group 3, shows not only the stage of degeneration in these animals as indicated by the kidney injury and the disturbance in the acid-base equilibrium of the blood, but also the second period in the intoxication, which is characterized by a partial restoration in the functional capacity of the kidney, and by an attempt to restore the normal acid-base equilibrium of the blood.

The response of this youngest group of animals to an intoxication by uranium is similar qualitatively to the response which has been obtained in Groups 1 and 2 of the older animals. The difference in the reaction of these younger animals to uranium is shown by a delay in the development of the kidney injury and by the fact that the alkali reserve of the blood does not show so rapid a depletion as was the case with the older groups of animals. A further study also shows that an animal of this group (Experiment 4, Table IV) may during the course of the intoxication show as marked evidence of kidney injury and as great a depletion in alkali reserve as did the older animals of Groups 1 and 2, yet following this degree of injury the younger animals (Group 3) are able to some extent to return to the normal, while with the older animals such a reparative reaction has not occurred.

In Experiments 3 and 4 (Tables III and IV) these younger animals have shown less albumin in the urine than has been the case with the older animals. During the first 24 hour period of the intoxication

these animals have shown a reduction in the elimination of phenolsulfonephthalein and a retention of blood urea. In these animals, however, the reduction in the elimination of the dye is not so rapid and the retention of blood urea is not so great as is the case with the older groups of animals.

In this group of young animals there is the usual association between the kidney injury and the reduction in the alkali reserve of the blood. This disturbance in the acid-base equilibrium of the blood shows a gradual development. In Experiment 4 (Table IV)

TABLE III.

*Prolonged Uranium Intoxication. Group 3, Experiment 3.*

| Day of experiment. | Age and weight of animal. | Water in 24 hrs. | Uranium nitrate per kg. | Urine in 24 hrs. | Albumin. | Glucose. | Blood urea. | Phthalein. | R. pH. | Carbon dioxide tension. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *cc.* | *mg.* | *cc.* | *gm.* | *per cent* | *per cent* | *per cent* | | *mm.* |
| 1 | 8 mos. 15.9 kg. | 500 | 0 | 418 | 0 | 0 | 0.012 | 58 | 8.1 | 40 |
| 2 | | 500 | 4 | 623 | 0.3 | 0.7 | 0.016 | 33 | 8.05 | 40 |
| 5 | | 500 | 0 | 310 | 1.0 | 1.19 | 0.024 | 22 | 8.0 | 40 |
| 8 | | 500 | 0 | 105 | 0.6 | 0.52 | 0.048 | 18 | 7.9 | 32 |
| 11 | | 500 | 0 | 230 | 0.2 | 0.3 | 0.070 | 10 | 7.9 | 31 |
| 14 | | 500 | 0 | 120 | 0.2 | 0.1 | 0.100 | 0 | 7.9 | 32 |
| 17 | 8 mos. 12.3 kg. | 500 | 0 | 503 | Tr. | 0 | 0.200 | 0 | 7.9 | 34 |
| 20 | | 500 | 0 | 700 | 0 | 0 | 0.206 | Tr. | 7.9 | 30 |
| 23 | | 500 | 0 | 700 | 0 | 0 | 0.208 | " | 7.9 | 30 |
| 26 | | 500 | 0 | 794 | 0 | 0 | 0.218 | 4 | 7.95 | 36 |
| 29 | | 500 | 0 | 628 | 0 | 0 | 0.180 | 8 | 7.95 | 38 |
| 32 | | 500 | 0 | 623 | Tr. | 0 | 0.188 | 10 | 7.95 | 38 |
| 35 | | 500 | 0 | 629 | 0 | 0 | 0.163 | 10 | 8.0 | 38 |

the greatest reduction in alkali reserve was 7.75, which was obtained on the 12th day of the experiment. In Experiment 3 the greatest reduction was 7.9. This reading was first obtained on the 8th day of the intoxication.

Judging by the percentage elimination of phenolsulfonephthalein, the degree of retention of blood urea, and the maximum reduction in the alkali reserve of the blood, we find that the height of the intoxication by uranium in these animals is reached between the 8th and 15th days of the experiment. Following this period, in this group of young

animals, the functional capacity of the kidney shows a beginning improvement and the alkali reserve of the blood shows less depletion. The histological study of the kidney during such a period of functional recuperation shows, in addition to the evidence of repair in the connective tissue element, a progressive epithelial regeneration.

In Experiment 4, Group 3, (Table IV), the height of the intoxication was reached on the 15th day of the experiment. At this stage

TABLE IV.

*Prolonged Uranium Intoxication. Group 3, Experiment 4.*

| Day of experiment. | Age and weight of animal. | Water in 24 hrs. | Uranium nitrate per kg. | Urine in 24 hrs. | Albumin. | Glucose. | Blood urea. | Phthalein. | R. pH. | Carbon dioxide tension. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *cc.* | *mg.* | *cc.* | *gm.* | *per cent* | *per cent* | *per cent* | | *mm.* |
| 1 | 11 mos. 12.58 kg. | 500 | 0 | 605 | 0 | 0 | 0.016 | 81 | 8.0 | 42 |
| 2 | | 500 | 4 | 619 | 0.75 | 0.81 | 0.028 | 56 | 7.95 | 38 |
| 3 | | 500 | 0 | 211 | 1.2 | 0.85 | 0.038 | 51 | 7.9 | 34 |
| 6 | | 500 | 0 | 189 | 0.4 | 0.27 | 0.077 | 31 | 7.9 | 34 |
| 9 | | 500 | 0 | 475 | Tr. | Tr. | 0.177 | 8 | 7.85 | 31 |
| 12 | | 500 | 0 | 192 | 0 | " | 0.180 | Tr. | 7.75 | 20 |
| 15 | | 500 | 0 | 397 | 0 | 0 | 0.297 | 0 | 7.75 | 24 |
| 18 | | 500 | 0 | 653 | Tr. | 0 | 0.183 | 24 | 7.8 | 27 |
| 21 | | 500 | 0 | 611 | " | 0 | 0.108 | 24 | 7.8 | 27 |
| 24 | 11 mos. 9.5 kg. | 500 | 0 | 459 | Tr. | 0 | 0.073 | 29 | 7.8 | 27 |
| 27 | | 500 | 0 | 561 | " | 0 | 0.082 | 35 | 7.85 | 30 |
| 30 | | 500 | 0 | 355 | 0.25 | 0 | 0.045 | 37 | 7.95 | 37 |
| 33 | | 500 | 0 | 283 | 0.6 | 0 | 0.120 | 21 | 7.9 | 36 |
| 36 | | 500 | 0 | 523 | 0.7 | 0.34 | 0.054 | 21 | 7.95 | 36 |
| 39 | | 500 | 0 | 525 | 0.2 | 0 | 0.061 | 16 | 8.0 | 40 |
| 42 | | 500 | 0 | 610 | 0.2 | 0 | 0.082 | 21 | 8.0 | 40 |
| 45 | | 500 | 0 | 531 | Tr. | 0 | 0.053 | 28 | 8.0 | 40 |
| 48 | | 500 | 0 | 566 | " | 0 | 0.065 | 28 | 8.0 | 40 |

of the experiment the animal gave a negative phenolsulfonephthalein test, the blood urea showed a retention of 0.297 per cent, and the alkali reserve of the blood had been reduced to 7.75. On the 18th day the animal had an elimination of phenolsulfonephthalein of 24 per cent, a reduction in the retention of blood urea to 0.183 per cent, and an increase in the alkali reserve of the blood to 7.8. During the further course of the experiment, which was terminated on

the 48th day, there occurred a gradual increase in the elimination of phenolsulfonephthalein, a decrease in the retention of blood urea, and an increase in the alkali reserve of the blood until the 30th day of the intoxication. At this period there occurred an unexplained relapse in the animal's condition. The phenolsulfonephthalein elimination dropped to 21 per cent and during the following 6 days went as low as 16 per cent. With this change in the ability of the kidney to eliminate the dye, the blood urea rose from a retention of 0.045 per cent to a retention of 0.120 per cent. The alkali reserve of the blood was reduced from 7.95 to 7.9. Following this period of secondary injury another phase of improvement developed, so that at the termination of the experiment on the 48th day the animal had an elimination of phenolsulfonephthalein of 28 per cent, a blood urea of 0.065 per cent, and a reserve alkali reading of 8.0.

The second experiment representative of this group of young animals (Experiment 3, Table III) shows a type of response similar to that of Experiment 4, which has been discussed in detail. Experiment 3 was terminated on the 35th day of the intoxication, 6 days after the functional response of the kidneys gave evidence of an improvement in the degree of intoxication and after the beginning restoration of the acid-base equilibrium of the blood.

The pathology of the kidney in the experiments of Group 3 has been studied at two periods of the intoxication. Experiments have been terminated and the kidneys obtained for study within 10 days after the functional observations showed the period of improvement in the animals to have been established. In other animals of the group the experiments were terminated after the stage of improvement had persisted for a longer period, 20 to 38 days. By such a selection of tissue not only have observations been made of the processes of repair in the kidney, but it has been possible to study these changes in connection with the reestablishment of the functional capacity of the kidney.

The kidneys obtained from animals in the early stages of improvement from the intoxication show no gross evidence of disease.

The histological study shows a late stage in the regeneration of tubular epithelium (Fig. 3). The regenerated epithelium remains of the flattened atypical type. The cytoplasm which stains deeply has

differentiated into cell units. The nuclei are hyperchromatic and of large size in proportion to the surrounding cytoplasm. Most of the granular detritus, the result of the early epithelial necrosis, has been removed from the newly lined tubules. In some of the tubules regeneration has failed to develop. Such tubules are filled with necrotic material. A diffuse formation of intertubular connective tissue has occurred which is in the cellular stage.

The vascular pathology has consisted in an invasion of the glomeruli by fibroblasts with a matting together of the capillary loops. The capsules of the glomeruli have shown an early thickening with a marked periglomerular cellular fibrosis (Fig. 4).

The kidneys obtained from animals late in the period of recovery from the intoxication have failed to show any gross change, such as an adherent capsule, scarring of the cortex, or any marked decrease in the proportionate relation between the cortex and medulla.

The histological study of this tissue has shown a later stage in the process of repair than was shown by tissue obtained from animals soon after a beginning recovery from the intoxication had been established. The sections have shown an increase in the number of tubules containing flattened, regenerated epithelium. Tubules with degenerating and necrotic epithelium are less numerous. The glomeruli show a later stage of fibrosis and the capsules show newly formed connective tissue fibers. The periglomerular and intertubular connective tissue has passed from the cellular stage to the stage of laying down connective tissue fibers (Fig. 5).

## DISCUSSION.

The experiments conducted in this investigation confirm the earlier work of Dickson, who demonstrated that uranium would produce in certain of the lower animals a chronic kidney injury comparable with certain of the chronic diffuse nephropathies of man. The experiments furthermore demonstrate the character and the severity of the functional disturbance associated with the various stages of the uranium intoxication. In these experiments it has been shown that the severity of the acute degenerative changes in the kidney is largely dependent upon the age of the animal. The older

animals develop a more rapid and a severer type of intoxication than is the case with the younger animals. The intoxication is characterized by a reduction in the alkali reserve of the blood and by the development of a kidney injury. The injury to the kidney is expressed functionally by the appearance of albumin in the urine, a reduction in the elimination of phenolsulfonephthalein, and by a retention of blood urea. The reduction in the elimination of the dye occurs as the first indication of renal injury. The reduction progressively increases with the severity of the degeneration in the kidney. The retention of blood urea is a somewhat later manifestation of kidney injury. A retention of blood urea may be deferred for 6 to 36 hours after the development of a marked reduction in the ability of the kidney to eliminate phenolsulfonephthalein. Subsequently, during the period of acute degeneration, the retention of blood urea shows a progressive increase.

Albumin has appeared in the urine of all the animals during the first 24 hours. Following its appearance the amount of albumin increases for the first 2 to 6 days of the experiment, and during this period the quantitative output of albumin has shown a relation with the renal functional tests. After this period, however, the amount of albumin in the urine rapidly decreases, while the reduction in the elimination of phenolsulfonephthalein and the retention of blood urea progressively increase. From this observation it would appear that quantitative determinations of the amount of albumin in the urine may give very imperfect information concerning the degree of renal injury.

All the animals intoxicated by uranium have shown a disturbance in the acid-base equilibrium of the blood, as indicated by a reduction in the alkali reserve and by a decrease in the tension of alveolar air carbon dioxide. The depletion in the alkali reserve has developed more rapidly and has shown a greater degree of reduction early in the experiments on the older animals than has been the case with the younger animals. The severity of the intoxication, as expressed by the degree of functional disturbance of the kidneys, has shown a parallel with the severity of the disturbance in the acid-base equilibrium of the blood.

A study of the intoxication in the animals of the various age groups shows that the older animals develop a type of kidney injury in which there is no attempt at regeneration, and therefore no re-establishment of renal function. In these animals there has failed to develop any attempt to restore the normal acid-base equilibrium of the blood. In the younger animals, however, epithelial regeneration does occur, and following this attempt at repair the functional capacity of the kidney improves and the depletion of the alkali reserve of the blood is in part restored. The earliest evidence of such functional restoration has consisted in a return of the ability of the kidney to eliminate phenolsulfonephthalein. Following this improvement the percentage retention of blood urea is decreased and still later the acid-base equilibrium of the blood returns towards the normal. In two of the younger animals during the period of recovery from the intoxication, the acid-base equilibrium of the blood was restored to the point of normality, yet in these animals the retention of blood urea remained high and the elimination of phenolsulfonephthalein was sufficiently low to indicate the existence of a severe grade of kidney injury.

## CONCLUSIONS.

1. Uranium nitrate is relatively more toxic for old animals than for young animals.
2. This relative toxicity is not only expressed in the old animals by a greater functional disturbance of the kidney, but is also shown by an inability on the part of these animals to repair the kidney injury and reestablish its functional capacity.
3. The intoxication in younger animals has been followed by a repair of the renal injury and a partial restoration of kidney function.
4. In these animals the processes of repair lead to the development of a chronic diffuse type of nephropathy in which the acid-base equilibrium of the blood may be maintained at the point of normality. In these animals renal functional tests indicate the presence of severe kidney injury.

## EXPLANATION OF PLATES.

### PLATE 26.

FIG. 1. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the animal of Experiment 1, Group 1 (Table I). It shows at *A* an acute swelling and vacuolation of the convoluted tubule epithelium. At *B* are shown tubules in which the epithelial degeneration has advanced to the stage of complete necrosis. At *C* is shown a junctional tubule distended with necrotic epithelial debris. Between the convoluted tubules at *D* there has occurred an extravasation of blood.

### PLATE 27.

FIG. 2. Camera lucida drawing, Leitz oc. 2, obj. $\frac{1}{12}$. Oil immersion. The figure is from the kidney of the animal of Experiment 2, Group 2 (Table II). It represents the different stages in the regeneration of a flattened atypical type of convoluted tubule epithelium. At *A* is shown a regenerated epithelial cell containing a mitotic figure. This tubule is partially lined by regenerated cells. The tubule also contains the remains of necrotic cell material. At *B* and *C* are shown tubules in the early stage of epithelial regeneration. At *D* is shown a tubule in a later stage of the process of epithelial regeneration. The large epithelial cells which are first regenerated are here flattening out to line the tubule with an atypical and less specialized type of epithelium, which is characterized by large hyperchromatic nuclei and a small amount of deeply staining cytoplasm.

### PLATE 28.

FIG. 3. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the animal of Experiment 3, Group 3 (Table III). It shows a later stage in the regeneration of tubular epithelium and the stage in the delayed uranium intoxication which is characterized by the formation of intertubular connective tissue. At *A* are shown convoluted tubules lined by a flattened type of epithelium. The cytoplasm has differentiated with the formation of definite cell boundaries. The necrotic material has been removed from the lumen of the tubules. At *B* are shown tubules filled with the remains of necrotic epithelium. No epithelial regeneration has developed in these tubules. At *C* is shown the early formation of cellular connective tissue.

### PLATE 29.

FIG. 4. Camera lucida drawing, Leitz oc. 2, obj. 6. This figure is taken from the kidney of the same animal as Fig. 3. It shows at *A* a glomerulus in an early stage of connective tissue infiltration. Connective tissue nuclei are numerous. The capillary loops have become obliterated and matted together. At *B* is shown an early thickening of the capsule of the glomerulus and a marked peri-

glomerular cellular fibrosis. At *C* are shown tubules lined with regenerated flattened epithelium. At *D* convoluted tubules may be seen in an early stage of degeneration. At *E* is shown a marked intertubular cellular fibrosis in which are several newly lined tubules.

PLATE 30.

FIG. 5. Camera lucida drawing, Leitz oc. 2, obj. 6. The figure is from the kidney of the animal of Experiment 4, Group 3 (Table IV). It shows an advanced stage of repair following the uranium intoxication which has resulted in the development of a chronic diffuse type of nephropathy. At *A* is shown the predominance of regenerated tubules, lined by flattened epithelium. At *B* is shown a shrunken glomerulus with a capsule thickened by the laying down of connective tissue fibers. At *C* the intertubular connective tissue has passed from the early cellular stage into the stage of connective tissue fiber formation.

# THE AGGLUTINATION REACTIONS OF THE MORGAN BACILLUS NO. 1.

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In the course of an investigation on the problem of soil pollution, bacilli having the cultural properties of the Morgan bacillus No. 1 were isolated from three polluted wells. The epidemiological significance of these findings is apparent provided that this organism really bears the relation to infectious diarrhea claimed for it by its discoverer. It was not feasible to enter into the question of etiology, but it has seemed desirable to compare the strains isolated from the polluted wells with some obtained from human sources. The resulting observations are of interest because they throw some light on the nature of this bacillus, which has been the subject of a great deal of discussion.

This organism was first described by Morgan in 1906. It belongs to the colon-typhoid group, but is peculiar in that it ferments only the hexoses with the production of gas. In the first epidemic of infectious diarrhea in which Morgan observed this organism he succeeded in isolating it from the stools of 40 out of 112 patients. In subsequent epidemics in England Morgan and his coworkers as well as Ross, Alexander, Lewis, and others, paid special attention to this type of bacillus. The reported findings are greatly at variance. Morgan isolated it in 54 per cent of the cases in 1905, 56 per cent in 1906, 16 per cent in 1907, and 53 per cent in 1908. Ross, Orr, and Alexander found this bacillus together with other non-lactose-fermenting bacteria, but claim that no one type was conspicuous. Alexander reports that during 1911 and 1912 the Morgan bacillus No. 1 was found in the stools of 4 per cent of normal and in 13.4 per cent of sick children examined. Lewis, on the other hand, in 1910 failed to isolate the organism from the stools of normal children, whereas he found it in 30 per cent of cases suffering from acute diarrhea. In 1911 he isolated the bacillus from 17 per cent of normal children and 70 per cent of the cases of diarrhea.

This peculiar organism has also been cultivated from sources other than human. Morgan and Ledingham isolated it from one cow out of eighteen examined; Lewis obtained it from five out of twenty mice. Cole isolated two strains from the intestinal contents of roaches.

The pathogenicity of the strains is variable. Both Morgan and Lewis proved that some strains were fatal for rats, mice, and rabbits when given *per os*. Neither Morgan nor Lewis was able to demonstrate the presence of specific antibodies in the sera of children from whom the bacilli were isolated.

The agglutination reactions of a large number of strains against different sera were reported by Lewis in 1912. He tested 242 strains from 167 cases, including those isolated from normal individuals, mice, cow, etc. against 16 sera. He found three main groups containing respectively 48, 17, and 8 per cent of the strains. There was no correlation between agglutination group and source. 11.5 per cent of the strains did not agglutinate with any of the sera. Lewis did not carry out any absorption tests.

It is evident from this brief review that the etiologic significance of the Morgan bacillus is by no means established. The work of Flexner and his coworkers, TenBroeck and Norbury, and Smillie leaves little room for doubt that in this country infectious diarrhea of infants is mainly caused by the dysentery bacilli. There remains, however, the possibility that the Morgan bacillus does play a minor part, since reports of its isolation frequently appear in the literature.

## EXPERIMENTAL.

*Strains Studied.*—The total number of strains tested was small but representative. In addition to the three strains isolated from polluted water there were fourteen others, obtained from a variety of sources. Three came from the Lister Institute, England; two were from the Institute of Public Health, Canada, and were originally sent there by Captain Fidlar from Mesopotamia. Three cultures were isolated in this country from stools of infants suffering from acute diarrhea. The others were either stock strains of unknown origin or recently isolated from other sources. Table I gives the number, source, and, when known, the year of isolation of the cultures used.

*Cultural Characters.*—Morphologically and culturally all the strains were identical and corresponded with the original description given by Morgan. They were all motile, Gram-negative bacilli, resembling the other members of the typhoid-dysentery group. They fermented the hexoses (dextrose, levulose, and galactose) with the production of acid and gas, but failed to ferment any of the other fermentable substances tested (mannite, maltose, lactose, saccharose, xylose,

arabinose, rhamnose, salicin, and dulcite). In semisolid agar containing glycerol and Andrade indicator they produced acid in the upper (aerobic) and not in the lower (anaerobic) part of the tube. All of them produced an abundance of indol in 24 to 48 hours in beef infusion broth. Gelatin was not liquefied. In their behavior toward the triphenylamine dyes they were more akin to the typhoid than to the dysentery bacilli.

TABLE I.

*List of Cultures Used.*

| Strain No. | Source. |
|---|---|
| M38a | Lister Institute, England. Origin not given. |
| M45a | " " " " " " |
| M33 | " " " " " " |
| M.J. | Institute of Public Health, Canada. Obtained from Captain Fidlar, Mesopotamia. |
| M.D. | Institute of Public Health, Canada. Obtained from Captain Fidlar, Mesopotamia. |
| 139 | Stock strain from the American Museum of Natural History, isolated from water. |
| T17 | Isolated, 1917 from the stool of a normal individual. |
| 57 | " 1917 " polluted well water. |
| 318 | " 1917 " " " " |
| 25 | " 1918 " " " " |
| M.R. | " 1916 " the intestine of a roach (Cole). |
| M.RX | " 1916 " " " " " " |
| 586 | " 1915 " " stool of an infant (diarrhea). |
| 692 | " 1915 " " " " " " |
| M.K. | Received from Professor Kendall. Origin unknown. |
| M.T. | " " Dr. TenBroeck. Isolated Aug., 1914, from a case of infant diarrhea in Boston. |
| S2 | Isolated, 1917, from polluted soil. |

*Pathogenicity.*—The pathogenic property of some of the strains was tested by feeding them to white mice and rats. The method of feeding was as follows: Veal broth containing 10 gm. of fresh veal to 100 cc. of the broth was used. The inoculated tubes were incubated for 48 hours at 37°C., and 10 cc. of these cultures fed to each animal by soaking the bread fed to them. Some of the animals were fed 10 cc. of culture on 2 consecutive days. None of the mice or rats became ill, although the bacilli were abundant in the feces for

several days after the feeding. The bacilli usually disappeared from the feces within 5 to 10 days after the feeding. Strains 692 and 586, most recently isolated from cases of acute diarrhea, and Strains 57, 318, and 139, obtained from polluted water, were employed in these tests.

*Agglutination Reactions.*—Agglutination tests were made with nine sera. The sera were prepared by injecting saline suspensions of

TABLE II.

*Agglutination Reactions of Morgan Bacilli in Different Sera.*

| Strain No. | Agglutination with Serum. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 692 | 586 | 139 | 318 | M. K. | T 17 | 33 | 45 | 38 |
| M38a | — | — | — | — | — | 1,280* | 320 | 2,560 | 2,560 |
| M45a | — | — | — | — | — | 2,560 | 640 | 2,560 | 640 |
| M33 | — | — | — | — | — | 1,280 | 1,280 | 1,280 | 2,560 |
| M.J. | 1,280 | — | 80 | — | — | — | — | — | — |
| M.D. | — | — | — | — | 2,560 | 2,560 | — | 40 | 80 |
| 139 | — | — | 1,280 | — | — | — | — | — | — |
| T17 | — | — | — | — | 320 | 2,560 | — | 2,560 | 640 |
| 57 | — | 40 | — | 80 | — | — | 40 | — | — |
| 318 | — | — | — | 2,560 | — | — | — | — | — |
| 25 | — | — | 1,280 | — | — | — | — | — | — |
| M.R. | — | — | 40 | — | 80 | 1,280 | — | — | — |
| M.RX | — | — | — | — | — | — | — | — | 160 |
| 692 | 1,280 | — | — | — | — | — | — | — | — |
| 586 | — | 1,280 | — | — | — | — | — | — | 80 |
| M.K. | — | — | — | — | 2,560 | 2,560 | — | 640 | — |
| M.T. | | | | | | | 160 | — | — |
| S2 | — | 1,280 | — | — | — | — | — | — | — |

* The numbers indicate the highest dilution at which agglutination occurred.

24 hour growths on agar slants. Five injections at 3 day intervals were usually given. The results are summarized in Table II. The numbers in the table indicate the highest dilution in which agglutination occurred. It is evident at a glance that the strains are highly diversified antigenically. The seventeen strains fall into six distinct groups.

*Absorption Tests.*—The heterogeneity of these strains is more strikingly shown by the absorption tests. Cultures T17 and M.K., for

example, cross-agglutinated in high dilutions, but their respective sera behaved differently towards the other strains. Absorption tests

TABLE III.

*Absorption of Agglutinins from Sera Produced against Various Strains of Morgan Bacilli.*

| No. of agglutinating culture. | Serum 38 absorbed with. | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Culture 38. | | | | | Culture 33. | | | | | Culture 45. | | | | |
| | 80 | 160 | 320 | 640 | 1,280 | 80 | 160 | 320 | 640 | 1,280 | 80 | 160 | 320 | 640 | 1,280 |
| 38 | ++ | + | − | − | − | ++ | ++ | ++ | + | + | ++ | ++ | ++ | ++ | + |
| 33 | + | ± | − | − | − | ++ | ++ | + | − | − | ++ | ++ | ++ | + | − |
| 45 | − | − | − | − | − | + | ± | − | − | − | ++ | ++ | + | − | − |
| T17 | − | − | − | − | − | + | ± | − | − | − | ++ | ++ | ± | − | − |
| | Serum 45 absorbed with. | | | | | | | | | | | | | | |
| | Culture 45. | | | | | Culture T17. | | | | | Culture 38. | | | | |
| 38 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 33 | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − |
| 45 | + | − | − | − | − | ± | − | − | − | − | ++ | ++ | ++ | + | − |
| T17 | + | − | − | − | − | ± | − | − | − | − | ++ | ++ | ++ | + | − |
| | Serum 33 absorbed with. | | | | | | | | | | | | | | |
| | Culture 33. | | | | | Culture 45. | | | | | | | | | |
| 33 | − | − | − | − | − | ++ | ++ | ++ | ++ | ++ | | | | | |
| 45 | − | − | − | − | − | − | − | − | − | − | | | | | |
| | Serum T17 absorbed with. | | | | | | | | | | | | | | |
| | Culture T17. | | | | | Culture M. K. | | | | | | | | | |
| T17 | | − | − | − | − | ++ | ++ | ++ | ++ | ++ | | | | | |
| M.K. | | − | − | − | − | − | − | − | − | − | | | | | |
| | Serum M. K. absorbed with. | | | | | | | | | | | | | | |
| | Culture M. K. | | | | | Culture T17. | | | | | | | | | |
| T17 | − | − | − | − | − | + | ± | − | − | − | | | | | |
| M.K. | + | − | − | − | − | ++ | ++ | ++ | ++ | ++ | | | | | |

showed that they were distinct types. Similarly, the sera produced against the English strains M38a, M45a, and M33, cross-agglutinated the heterologous strains in high dilution, but absorption tests

showed that the three organisms were antigenically different. The seventeen strains are thus separated into ten distinct antigenic types without any correlation as to source. The results of the absorption tests are given in Table III.

## SUMMARY AND CONCLUSION.

The study of the cultural and agglutinating reactions of seventeen strains of Morgan bacilli is reported. The cultures were obtained from different sources. Culturally all the strains were identical. Antigenically they were highly diversified. The sera produced against some strains have marked agglutinating power for other strains, but absorption tests showed that the cross-agglutinations were often due to group agglutinins. While no conclusion can be drawn regarding the pathogenic significance of this bacillus, the wide diversities of antigenic properties raise the question as to the specific relationship of the various cultures met with as well as their relation to a definite class of pathological processes in man.

## BIBLIOGRAPHY.

Alexander, D. M., *Great Britain Rep. Local Gov. Bd., Suppl. Med. Officer*, 1911–12, xli, Appendix B, 288; 1912-13, xlii, Appendix B, 384.

Bacteriological and clinical studies of the diarrheal diseases of infancy with reference to the *Bacillus dysenteriæ* (Shiga) from The Rockefeller Institute for Medical Research, edited by Flexner, S., and Holt, L. E., in Studies from The Rockefeller Institute for Medical Research, New York, 1904, ii, 1.

Cole, C. L., Personal communication.

Lewis, C. J., *Great Britain Rep. Local Gov. Bd., Suppl. Med. Officer*, 1910–11, xl, Appendix B, 314; 1911–12, xli, Appendix B, 265.

Morgan, H. de R., *Brit. Med. J.*, 1906, i, 908; 1907, ii, 16.

Morgan, H. de R., and Ledingham, J. C. G., *Proc. Roy. Soc. Med., London, Epidemiol. Sect.*, 1908–09, ii, 133.

Orr, T., *Great Britain Rep. Local Gov. Bd., Suppl. Med. Officer*, 1910–11, xl, Appendix B, 374.

Ross, S. M., *Great Britain Rep. Local Gov. Bd., Suppl. Med. Officer*, 1910–11, xl, Appendix B, 347.

Smillie, W. G., *Am. J. Dis. Child.*, 1917, xiii, 337.

TenBroeck, C., and Norbury, F. G., *Boston Med. and Surg. J.*, 1915, clxxiii, 280; 1916, clxxiv, 785.

# EDEMA OF THE LUNGS AS A CAUSE OF DEATH.*

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(Received for publication, April 3, 1919.)

Edema of the lung is a frequent terminal event in the course of many diseases, both acute and chronic, and in such cases is commonly interpreted as the immediate cause of death. It constitutes, as is well known, a striking feature in poisoning by most of the noxious gases in modern warfare. It is, therefore, not surprising that in the clinical and pathological reports of these fatal gas cases, death should be regularly attributed to pulmonary edema.

The question as to how edema of the lungs may bring about death has long been discussed. The view generally accepted is that the fluid in the pulmonary alveoli interferes mechanically with gaseous interchange and that when this interference with respiration passes a certain critical point, the patient dies of asphyxia; that is, he drowns in his own fluid. It is not our purpose in this paper to discuss the question as to how edema of the lungs may cause death, but rather the fundamental problem of whether the mere accumulation of fluid in the lung is of itself a serious matter.

During the past 2 years we have had the opportunity of studying the effects of practically all the commonly used war gases under laboratory conditions. About 3,000 dogs and an equal number of other animals have been exposed to gas and then observed clinically and at autopsy. Particular attention has been given to the pulmonary edema, which, as in man, is a fairly constant and, in many cases, a striking phenomenon of the gassed state. These observations, together with the results of some experiments upon what

* This work is an incidental result of more detailed studies in the pathology of war gases which were conducted under the auspices of the Bureau of Mines, the Chemical Warfare Service, and the Office of the Surgeon General, U. S. Army.

may be termed an artificial pulmonary edema produced by filling the lungs of a normal dog with an isotonic salt solution, have led us not only to question the importance of pulmonary edema *per se* as a cause of death, but to conclude that edema of the lungs in general is merely an indicator of some underlying disorder and is rarely, if ever, directly responsible for the death of the patient or animal. These observations are summarized in the following paragraphs.

Animals which die acutely from exposure to any of the gases of the respiratory irritant group, such as chlorine and phosgene, show at autopsy varying degrees of edema of the lungs. Although this is regularly well marked in certain species, dogs for example, there are wide individual variations. In other species, rats and guinea pigs for example, it may be a relatively inconspicuous feature in spite of the fact that these animals are particularly susceptible to the gas.

Likewise dogs which are killed before the action of the gas reaches its maximum effect show striking differences in the amount of fluid in the lungs, and these differences do not harmonize with the variations in the symptoms manifested by the animals. Furthermore, many dogs which pass successfully the critical 48 hour period and are classed as "recovered" often show, when killed, edema of the lungs of greater degree than other dogs of the same experiment which succumbed.

The increased concentration of the blood in the gassed animal, which some investigators regard as a reliable index of the condition of the animal and as a guide to therapy, does not correspond necessarily in any measure with the degree of pulmonary edema present.

The lungs of a normal animal may be filled with isotonic salt solution, thus producing an artificial edema comparable with that found in the gassed state.

Several of the propositions set forth require elaboration and discussion. They are considered in detail below.

*Variations in the Degree of Pulmonary Edema in Gassed Animals.*

The gross and microscopic picture of the lungs associated with acute gassing has been so thoroughly described in the papers published from this laboratory and elsewhere as to need no repetition

here.[1] As pointed out above, edema of the lungs in dogs is generally well marked, and with the associated congestion constitutes the most striking feature of the autopsy findings. However, among individual dogs dying in the acute stage the degree of edema has been found to vary within relatively wide limits. These differences are obvious to the naked eye, but in order to make satisfactory comparisons, free from the personal equation, we adopted the following method for measuring the degree of edema. This method, which is based on the comparative weights of the lung and empty heart, was suggested and used by Barcroft in the investigations carried on by the British Research Committee.

*Method.*—The lungs are weighed with the trachea attached, cut to uniform length, and clamped to prevent the escape of edema fluid. The heart is also trimmed uniformly and completely emptied. The normal ratio of the lung weight to the heart weight was obtained by taking an average of sixteen normal animals. This normal ratio was found to be 1:30. Barcroft's higher figure (1:50) is probably the result of a difference in the method of trimming the organs. In gassed dogs we have divided the lung weight by the heart weight, and this quotient by the normal ratio, 1:30. We have termed the resulting figure the "edema index." It represents the percentage increase in lung weight over the normal. This method has been objected to by Eyster[2] who insists that the dried weight method is much more reliable. There are two of these dried weight methods; one, used by some of the French investigators, in which a typical slice of the lung is weighed wet and then after drying, and the second, the method used by Eyster and his assistants,[2] in which the entire lung is dried, the proportion between the wet and the dried specimen indicating the degree of edema. The first method is obviously open to large errors. The second method has the serious drawback of making it impossible to study the lung grossly or microscopically. We have compared the latter method with our more

[1] Winternitz, M. C., and collaborators, Collected studies on the pathology of war gas poisoning, New Haven, 1919; Anatomical changes in the respiratory tract initiated by irritating gases, *Mil. Surg.*, 1919 (in press).

[2] Eyster, J. A. E., Report of the Chemical Warfare Medical Committee, Medical Research Commission (unpublished).

simple lung-heart ratio method in a series of six dogs, and have found that the two methods give results which are approximately the same (compare Columns 9 and 13 in Table I).

TABLE I.

*Relation of the Edema of Lung and the Concentration of Blood in Gassed Animals.*

| Dog No. and breed. | Gas. | Concentration per liter. | Body weight. | Weight of empty heart. | Weight of wet lung. | Weight of dry lung. | Ratio of wet-dry lung. | Ratio of weight of lung-heart. | Red blood corpuscles before gas. | Red blood corpuscles before death. | Percentage increase of red blood corpuscles. | Edema index. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *mg.* | *kg.* | *gm.* | *gm.* | *gm.* | | | | | | |
| 1. Hound. | Phosgene. | 82 | 14.0 | 155 | 715 | | | 4.60 | 5,148,000 | 7,936,000 | 54 | 3.53 |
| 2. " | " | 80 | 18.5 | 159 | 760 | | | 5.40 | 6,068,000 | 10,870,000 | 79 | 3.42 |
| 3. Mastiff. | " | 75 | 26.0 | 170 | 915 | | | 5.40 | 8,400,000 | 10,808,000 | 28 | 4.15 |
| 4. Hound. | " | 82 | 17.8 | 154 | 822 | | | 5.34 | 7,172,000 | 7,640,000 | 7 | 4.10 |
| 5. Collie. | " | 99 | 17.8 | 177 | 570 | | | 3.22 | 6,192,000 | 11,260,000 | 82 | 2.48 |
| 6. Mongrel. | " | 102 | 6.4 | 53 | 320 | 27.5 | 11.7 | 5.93 | 8,280,000 | 10,760,000 | 30 | 4.56 |
| 7. " | " | 112 | 8.6 | 92 | 400 | 37.0 | 10.8 | 4.35 | 9,385,000 | 9,824,000 | 5 | 3.34 |
| 8. " | " | 109 | 11.0 | 93 | 375 | 38.0 | 9.8 | 4.04 | 7,520,000 | 8,432,000 | 12 | 3.11 |
| 9. " | " | 109 | 6.8 | 69 | 219 | 24.5 | 8.9 | 3.16 | 9,848,000 | 9,055,000 | | 2.44 |
| 10. Setter. | Chloropicrin. | 949 | 13.6 | 132 | 631 | 61.0 | 10.3 | 4.77 | 8,040,000 | 12,120,000 | 51 | 3.68 |
| 11. Mongrel. | Chloropicrin. | 840 | 9.5 | 103 | 600 | 46.0 | 13.1 | 5.84 | 6,276,000 | 6,458,000 | 3 | 4.50 |
| 12. Bull. | Chloropicrin. | 887 | 13.6 | 138 | 684 | 66.0 | 10.3 | 4.75 | 7,176,000 | 9,068,000 | 23 | 3.66 |

The dogs were killed 8 to 13 hours after exposure to phosgene or chloropicrin. Comparison of the figures in the last two columns (edema index and percentage increase in red blood cells) shows that no parallelism exists between the degree of pulmonary edema present and the blood concentration.

In using the lung-heart ratio method of estimating the amount of edema fluid present, we have found that the edema index in a series of dogs gassed under similar conditions varies within relatively wide limits. For example, among 50 dogs dying after exposure to phosgene the edema index ranged from 1.73 to 4.60, and in another series gassed with chloropicrin the extremes were 1.65 and 4.22.

In order to throw further light on the question of the significance of the degree of edema, the following experiment was done. Eight

dogs were gassed with phosgene (concentration 80 to 90 mg. per liter for 30 minutes). Four of the dogs died in from 10 to 15 hours. The remaining four were killed by chloroform. It was found that the average edema index of the four dogs that died was practically the same as that of the four that were killed. It was found also that many of the dogs which passed successfully through the critical 48 hour period and were then placed in the "recovered" group showed, if killed at this stage, a high edema index, often exceeding that of the dogs which had succumbed. It may be stated incidentally that these "recovered" dogs showed no symptoms other than occasional coughing and a slight sluggishness.

Still further evidence of the subsidiary part of edema as the cause of death after inhalation of irritating gases is found in the comparative effects of a gas such as phosgene on animals of different species.

A series of experiments was performed in which a number of different kinds of animals were exposed in the same chamber for 30 minutes to a concentration of 0.27 mg. per liter of phosgene. The time of survival varied as indicated in Table II.

TABLE II.

| Species. | Time of survival. |
|---|---|
| Monkey | 3 hrs., 30 min. |
| Guinea pig | 4 " 30 " |
| Rat | 5 " |
| Rabbit | 11 " 30 min. |
| Mouse | Killed after 12 hrs. |
| Dog | " " 12 " |
| Goat | " " 12 " |

The lesions produced in these animals by inhalation of phosgene are essentially alike. In the monkey and goat, for example, which represent the two extremes of susceptibility after exposure to the same concentration, lesions of the lung vary in degree but not in character. The species variation, evidenced by the length of the survival after gassing, in animals which have been killed or have died, may be expressed in part by the rate of development of the pulmonary edema. On the other hand, with some animals (monkey,

guinea pig), the first to succumb to a given concentration show less pulmonary edema than those that survive longer (dog, goat). This is evidence that the edema is in itself not the cause of death but simply one manifestation of a more important underlying change.

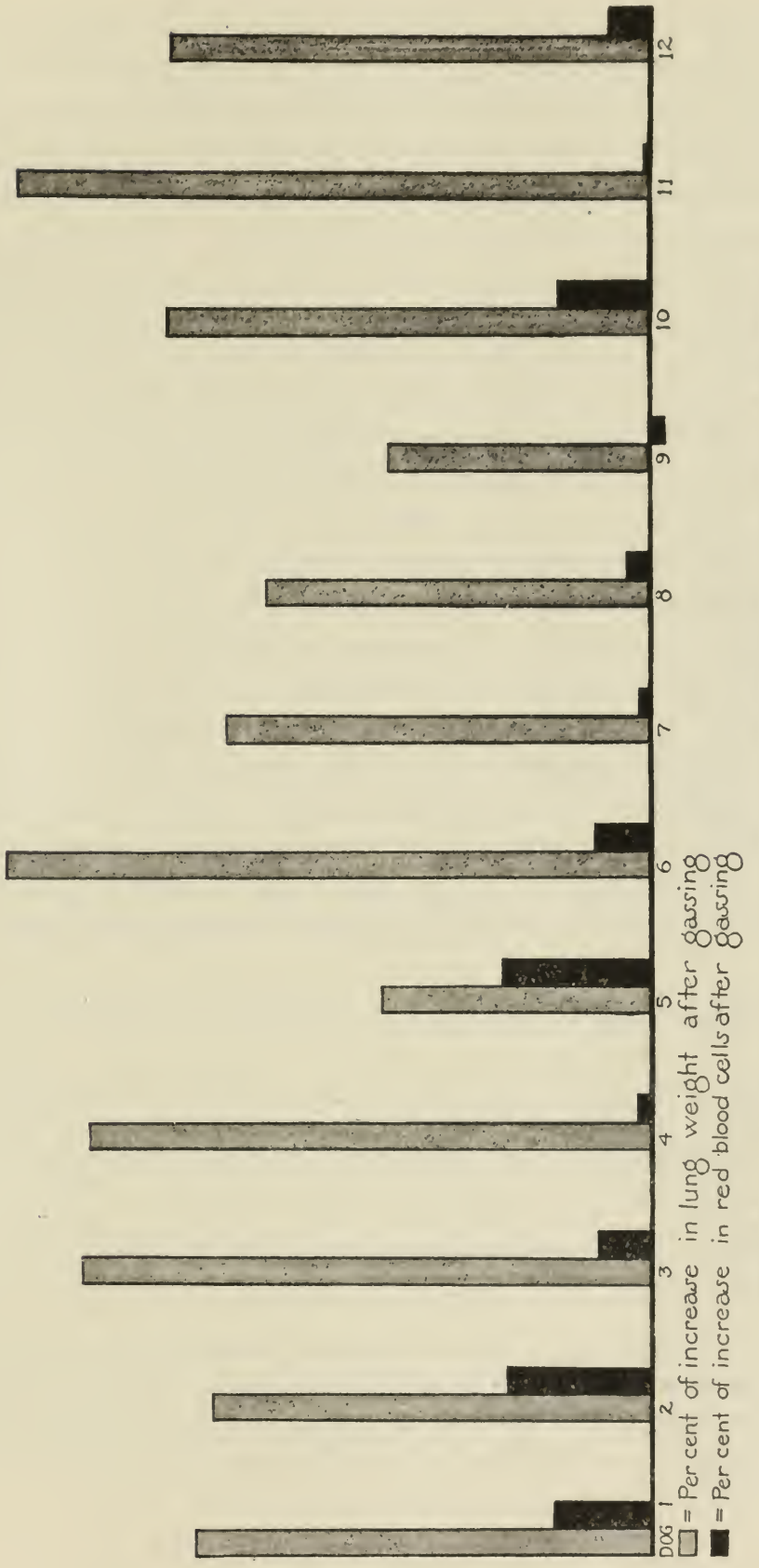
While the pulmonary edema develops more rapidly the more susceptible the species (Table II) the most susceptible show at death less edema than the more resistant ones. This is an indication of the importance of the time interval in the production of the edema.

*Relation of the Concentration of Blood and Edema of the Lung after Inhalation of Gas.*

Underhill[3] has found that dogs exposed to phosgene, chlorine, or chloropicrin show after a few hours (the time varies with different gases and with individual animals) a well marked increase in the concentration of the blood. Similar changes in the blood of gassed soldiers have been demonstrated repeatedly. The formed elements of the blood as well as the inorganic salts share in the change. Inorganic salts, however, do not follow the same course as the erythrocytes. The result is a marked increase in blood viscosity. Underhill and his assistants have used this blood change as an index of the condition of the gassed animals, and upon it have worked out a method of therapy, the essentials of which are bleeding and subsequent dilution of the residual blood with isotonic salt solution. In applying this method of treatment, which, it may be stated has definitely reduced the mortality among experimentally gassed dogs, it has been assumed that the concentration of the blood is due to the escape of blood serum into the lungs, and that, therefore, the increased viscosity of the blood may be taken as a rough index of the degree of pulmonary edema.

In order to determine whether or not these two phenomena, blood concentration and pulmonary edema, are directly related, the following experiment was carried out. Twelve dogs were gassed, nine with phosgene and three with chloropicrin, the duration of exposure and concentration being such as would be fatal to a majority of dogs exposed. The dogs were killed with chloroform about 10 hours after

[3] Report to be published in the near future.




TEXT-FIG. 1. Comparison of pulmonary edema and blood concentration after gassing.

the exposure to the gas; that is, as soon as the majority began to show serious symptoms. A red blood cell count was made before gassing and again just before the animal was chloroformed, since it has been shown that this is a reliable method for estimating the degree of blood concentration. The degree of pulmonary edema found was determined by the method described above. The figures for the increase in blood concentration and the edema index are recorded in Table I with other data. The results are also graphically shown in Text-fig. 1. It is seen that under the condition of these experiments no parallelism exists between the amount of fluid present in the lung and the degree of concentration of the peripheral blood. It is noteworthy that in one case in which there was a well marked edema of the lung, an actual reduction of blood concentration was found.[4] This experiment does not indicate that the loss of fluid from the blood may not have taken place by way of the lungs and the mouth, but, in our opinion, it does show conclusively that the change in the blood does not serve as an indicator of the amount of fluid present in the lung at any given moment. It suggests further that a therapy guided by the viscosity of the blood cannot be assumed to have any influence on the pulmonary edema, and that the beneficial results obtained by such therapy are probably in no way referable to a change in the fluid content of the lung, which of itself is of secondary importance, as will be emphasized in the following paragraph.

### *Artificial Edema of the Lungs. Pulmonary Irrigation.*

Winternitz and Smith[5] have shown that the lung is much less susceptible to the introduction of fluid than has been generally supposed. Repeated experiments have demonstrated that the lungs can be entirely flooded through the bronchi with isotonic salt solution and that this process of irrigation can continue for at least 2 hours with the introduction of 6 liters of fluid without causing any evident

[4] There is a dilution of the blood, as shown by Underhill, which precedes its concentration with phosgene poisoning. These changes of blood concentration may vary somewhat in duration, etc., and explain the charted findings above.

[5] Winternitz, M. C., and Smith, G. H., Sir William Osler Anniversary Volume, New York, 1919.

harmful changes in bodily well-being or any subsequent serious microscopic lesions in the lung tissue. By means of the use of colored solutions it has been shown that the fluid introduced actually passes throughout the lung, bronchi, bronchioles, and alveoli and does not simply flow back through the trachea without entering the lung.

# ETIOLOGY OF YELLOW FEVER.

## I. Symptomatology and Pathological Findings of the Yellow Fever Prevalent in Guayaquil.

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Plates 31 to 34.

(Received for publication, March 24, 1919.)

In this paper it is proposed to describe the clinical features and pathological changes observed in yellow fever cases admitted to the Yellow Fever Hospital in Guayaquil.[1] It may be stated at the outset that in an analysis of 172 cases no clinical or pathological facts were brought to light which had not been described by previous students of yellow fever in Guayaquil[2] or elsewhere. The yellow fever cases occurring in Guayaquil present no special feature; the disease is classic in all its aspects. For the sake of clearness the general clinical features and the individual symptoms of the disease will be described separately.

[1] This hospital is under the direction of Dr. Wenceslao Pareja. Dr. Pareja not only pointed out many interesting clinical features but also performed autopsies for the members of the Yellow Fever Commission during their sojourn in Guayaquil. I am greatly indebted to Dr. Pareja for his cooperation in my investigations and to Dr. Charles Elliott of the Commission for permission to use some of his clinical notes made on about half the total number of cases coming under my observation during my stay. The Yellow Fever Commission of the International Health Board was composed of Dr. Arthur I. Kendall, Dr. Charles A. Elliott, and Mr. Herman Edward Redenbaugh of Northwestern University Medical School, Chicago; Dr. Mario Lebredo of Las Animas Hospital, Havana, Cuba; and Dr. Hideyo Noguchi of The Rockefeller Institute for Medical Research, New York.

[2] Strong, P. P., Tyzzer, E. E., Brues, C. T., Sellards, A. W., and Gastiaburu, J. C., Harvard School of Tropical Medicine, Report of the first expedition to South America, 1913, Cambridge, 1915, 180–200.

## *Clinical Features.*

### *General Symptomatology.*

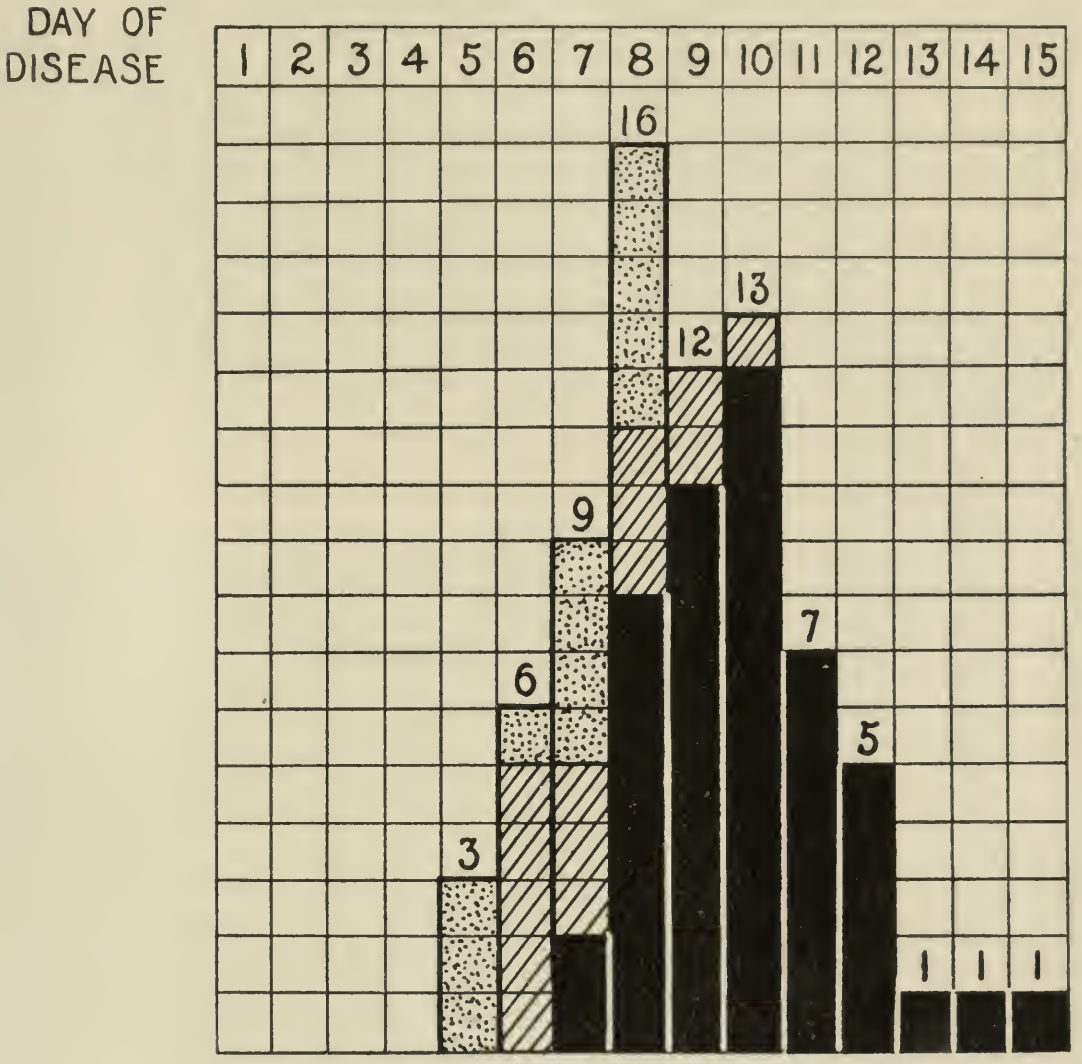
The incubation period varies from 3 to 6 days. It is difficult to determine the maximum. There may be prodromal symptoms for 1 or 2 days, but the onset is usually sudden, ushered in by fever, with or without chills. The patient feels gravely ill and in most cases goes to bed at once, with severe headache, pains in the loins, and anorexia; myalgia of the back, neck, and limbs is often intense, especially on pressure. Nausea and vomiting are frequently present. Insomnia and prostration follow. There is almost always tenderness in the epigastric region which gradually becomes aggravated as the disease progresses. The tongue has a heavy white coat with red tip and edge; later it may become brownish and dry. A peculiar, cadaveric-like odor emanates from the mouth. The gums are congested, swollen, and show a tendency to bleed on pressure. There is great thirst. The conjunctivæ are markedly suffused, becoming yellowish on the 2nd to 3rd day, sometimes with a few ecchymoses on the 6th to 7th day. The icterus of the conjunctivæ increases in the several days following and may persist 2 to 3 weeks in severe cases, although in milder ones it disappears in about 7 days. The black vomitus may occur on the 1st day or as late as several days after onset, or it may only be found in the stomach at autopsy. The skin is usually dry and icteric, and the patient suffers from intense epigastric pain. The gums may bleed profusely at this stage. The urine is reduced in volume; in many cases there is anuria for a day; the urine is dark, greenish, or brown, with abundant albumin and casts. Epistaxis occurs in many cases. Hiccoughs and other nervous symptoms (delirium, coma, convulsions) due to uremia and cholemia are frequent. Death may occur between the 4th and 9th days, rarely earlier or later.

### *Individual Symptoms.*

*Fever.*—The fever is very high for 1 to 2 days, reaching 39–41°C., then drops to about 38°C. and may persist from 3 to 8 days. On the average the temperature drops to 37° or even to 36°C. after 8 days and may continue to be subnormal for several days.

The relation between the temperature and the severity of the infection among those who recovered is interesting.

*Recovered Cases.*—There were 74 cases of yellow fever which could be analyzed. These cases came under our observation at different




TEXT-FIG. 1. 74 non-fatal cases analyzed according to defebrescence and severity of the disease. The light shaded area signifies mild, the cross-hatching moderately severe, and the solid black severe cases.

stages of the disease, ranging from the 2nd to the 8th day. The temperature in early cases (2nd, 3rd, 4th days) was usually very high, the majority being over 40°C., while those admitted later (5th to 8th days) had a temperature of 39°C. or below. The abate-

ment of fever to normal, or 37°C., occurred as convalescence was established. In the majority of instances in which the attack was mild or of moderate severity the temperature returned to normal on the 5th and 6th days. Those whose temperature returned to 37°C. on the 7th day were cases of moderate severity, though a few mild and severe cases had similar febrile reactions. The cases in which the temperature returned to 37°C. on the 8th, 9th, or 10th day were nearly all severe, and all those in which the normal was not reached until the 11th day or later were severe. The deduction from

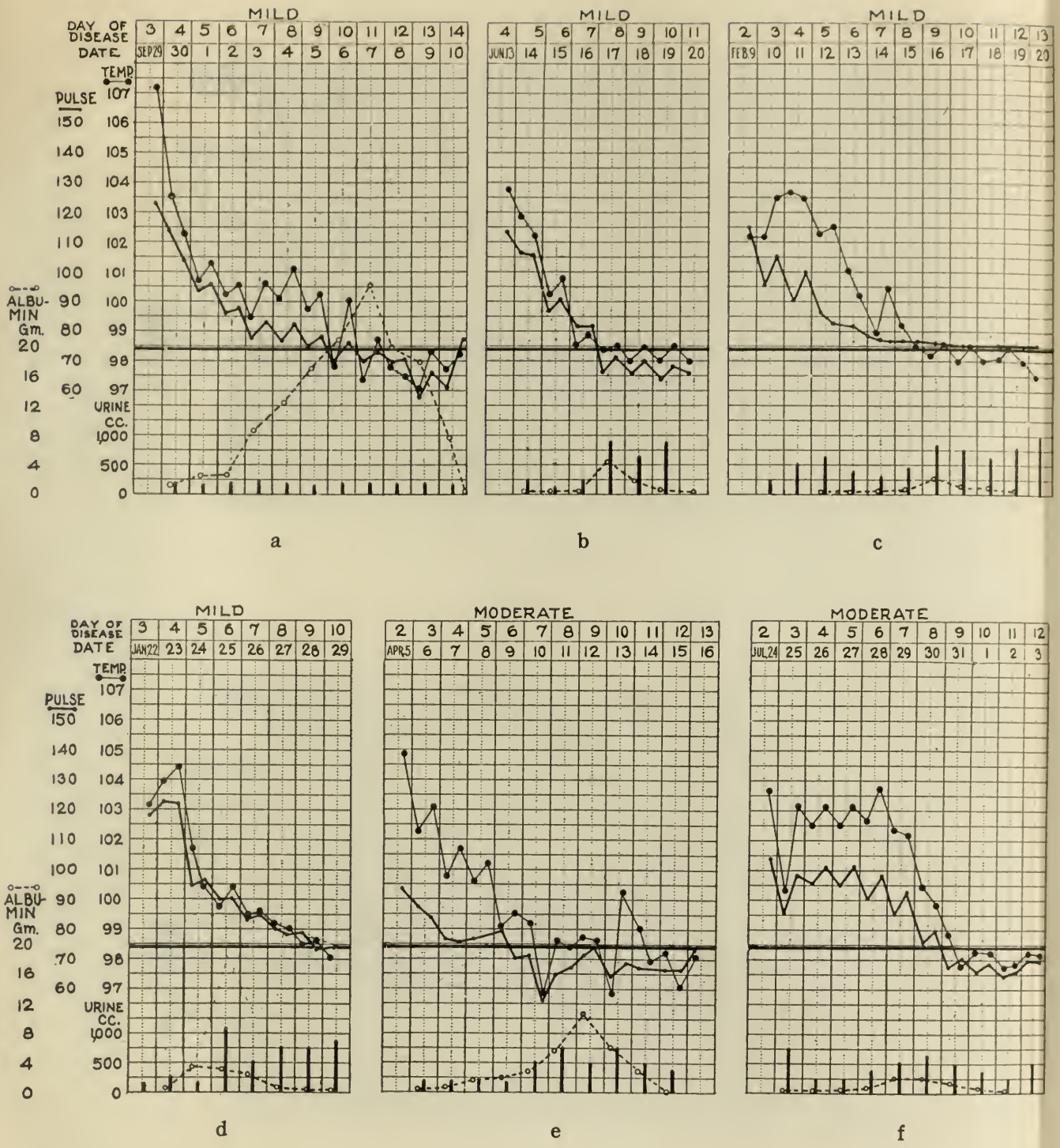
TABLE I.

*Relation between Time of Defebrescence and Severity of the Disease.*

| Day of disease when temperature reached 37°C. | Mild. | Moderate. | Severe. | Total. |
|---|---|---|---|---|
| .5 | 3 | | | 3 |
| 6 | 1 | 5 | | 6 |
| 7 | 4 | 3 | 2 | 9 |
| 8 | 5 | 3 | 8 | 16 |
| 9 | | 2 | 10 | 12 |
| 10 | | 1 | 12 | 13 |
| 11 | | | 7 | 7 |
| 12 | | | 5 | 5 |
| 13 | | | 1 | 1 |
| 14 | | | 1 | 1 |
| 15 | | | 1 | 1 |
| Total..... | 13 | 14 | 47 | 74 |

these observations is that the milder the attack the sooner the temperature returns to normal, and *vice versa*. In mild but undoubted cases of yellow fever the earliest date on which the normal has been attained is the 5th day of the disease (4 per cent). The bulk of the cases reached the normal on the 7th (12 per cent), 8th (20 per cent), 9th (16 per cent), or 10th (17 per cent) day. Text-fig. 1 and Table I show the distribution of cases from the standpoint of the time of defebrescence and severity of the disease.

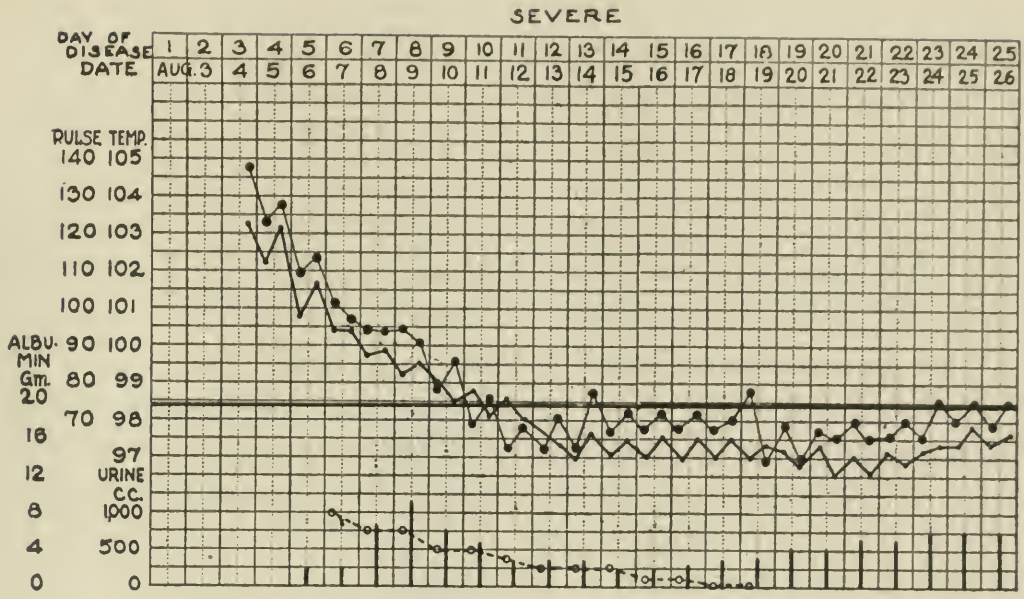
It was found that in the cases which were admitted to the hospital from the 2nd, 3rd, or 4th day of the disease there are several instances in which the temperature registered higher the following



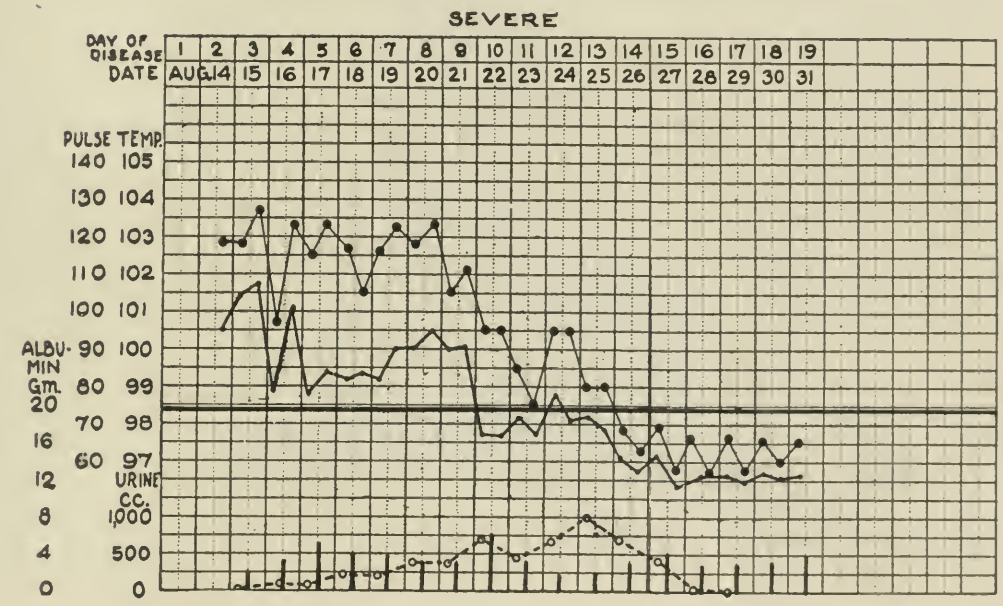

TEXT-FIG. 2, *a* to *f*. Mild and moderate cases of yellow fever. (*a*) Case 35. Age 25 years. (*b*) Case 36. Age 21 years. (*c*) Case 37. Age 22 years. (*d*) Case 38. Age 20 years. (*e*) Case 39. Age 18 years. (*f*) Case 9. Age 19 years.








a




b

TEXT-FIG. 3, *a* and *b*. Severe cases of yellow fever. (*a*) Case 19. Age 25 years. (*b*) Case 8. Age 21 years.

day than at the time of admission or that in some instances there was a distinct remission on the 2nd or 3rd day of the disease. Again in rare instances a relatively high fever (39–39.6°C.) lasted several days. The rapidity of lysis is somewhat variable in the different cases, but the drop in the curve is rather steep. When the temperature reaches 37°C. it usually goes further down, even as low as 36°C. within a few days and may remain subnormal for several days before it attains the normal permanently. Text-figs. 2, *a* to *f*, and 3, *a* and *b* illustrate these points.

*Fatal Cases.*—A study of the temperature in relation to fatal cases seemed important to an understanding of the clinical features of this disease. Among the records for the year (1918) I was able to utilize 66 cases of persons dying of yellow fever (Table II and Text-fig. 4). The bulk of deaths occurred on the 5th, 6th, and 7th days, but especially on the 5th and 6th. On the 4th and 8th, 9th, and 10th days the death rates were the same, being about one-third of those for the 5th and 6th and one-half of those for the 7th day. Death seldom occurred on the 2nd or 3rd day or after the 11th day of disease.

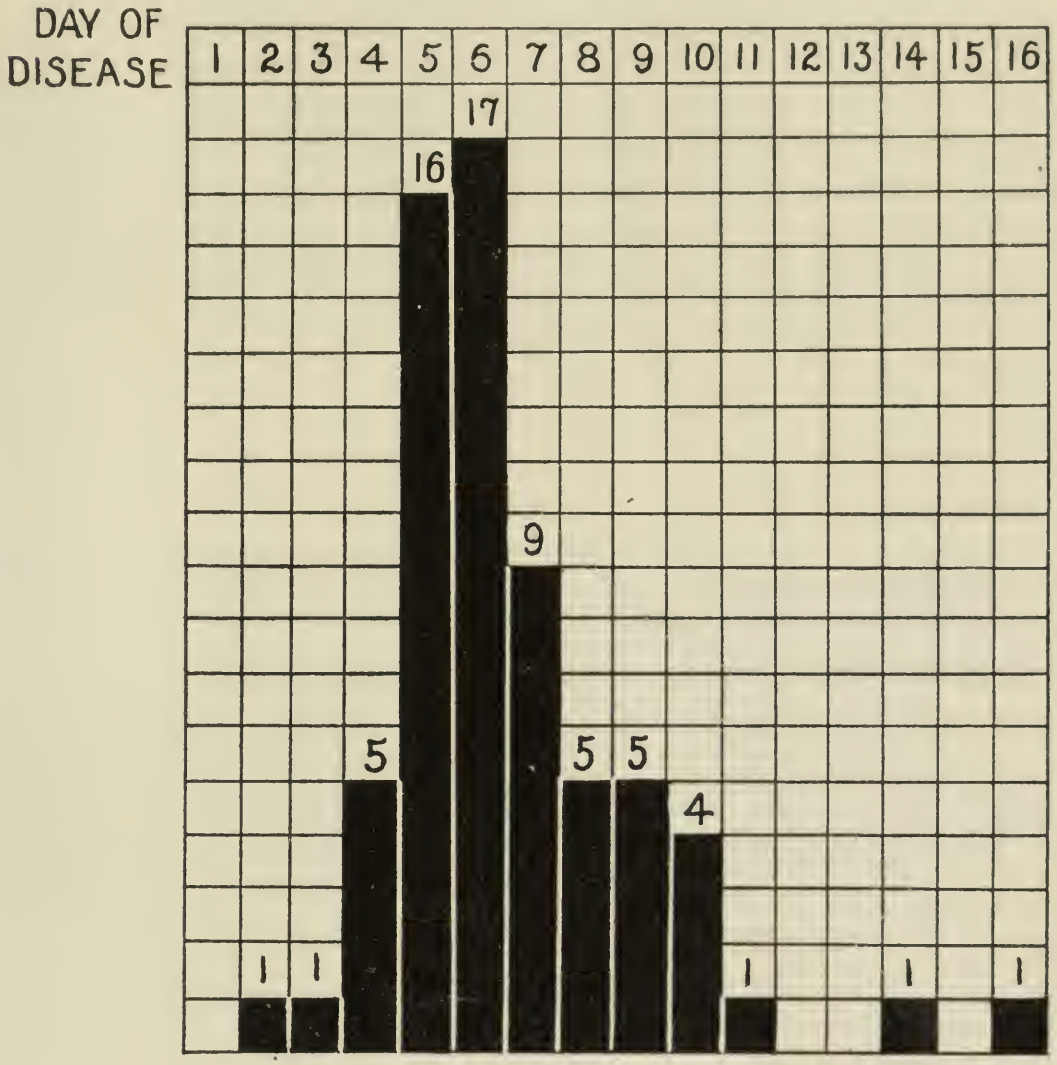
TABLE II.

*Fatal Cases.*

| Day of disease on which death occurred. | No. of cases. |
|---|---|
| 2nd | 1 |
| 3rd | 1 |
| 4th | 5 |
| 5th | 16 |
| 6th | 17 |
| 7th | 9 |
| 8th | 5 |
| 9th | 5 |
| 10th | 4 |
| 11th | 1 |
| 14th | 1 |
| 16th | 1 |
| Total | 66 |

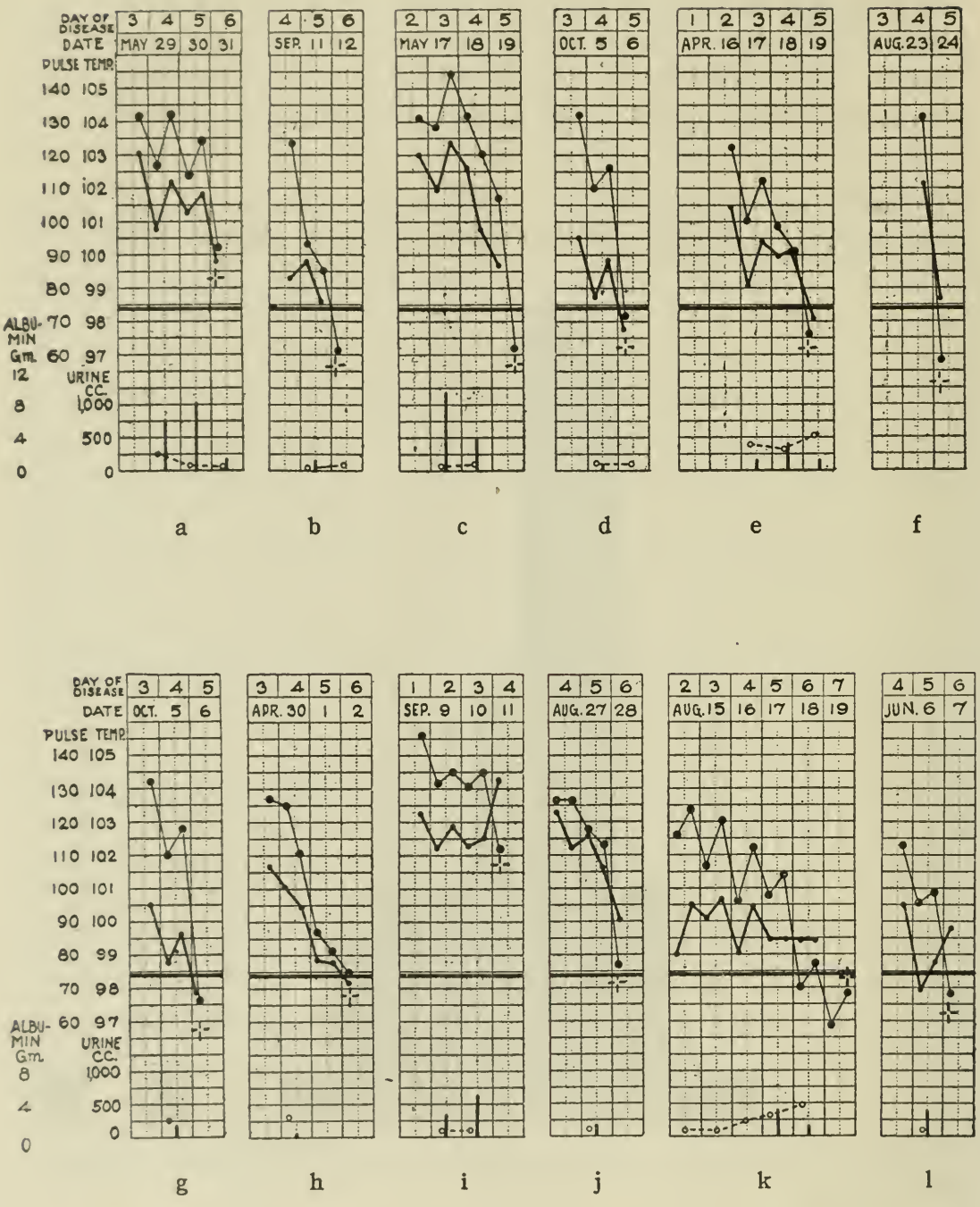
Total No. of cases.....140
No. recovered..... 74
" died..... 66 (47.1 per cent).

The temperature curves of these fatal cases (Text-fig. 5, *a* to *l*) are noteworthy as showing the rather rapid fall of temperature towards death. The initial fever in these cases was very high when observed during the early stage of the disease (above 40°C.), and some showed




TEXT-FIG. 4. The chart shows the day of disease on which death occurred in 66 fatal cases.

nearly 40°C. even on the 4th or 5th day. Death took place with a temperature as high as 39°C. in some and as low as 36°C. in others. In a few instances the temperature fell to about 37°C. or even 36° or 35°C. on the day of death but registered a sudden rise to 38°C., and in one case to 40.6°C., just before death.




TEXT-FIG. 5, *a* to *l*. Fatal cases of yellow fever. (*a*) Case 40. Age 28 years. (*b*) Case 41. Age 24 years. (*c*) Case 42. Age 20 years. (*d*) Case 43. Age 19 years. (*e*) Case 44. Age 28 years. (*f*) Case 45. Age 25 years. (*g*) Case 46. Age 18 years. (*h*) Case 47. Age 18 years. (*i*) Case 48. Age 13 years. (*j*) Case 49. Age 11 years. (*k*) Case 10. Age 26 years. (*l*) Case 50. Age 24 years.

The analysis of the febrile reaction of man to yellow fever infection becomes important in a discussion of the febrile reaction of certain experimental animals when inoculated with the blood or organ emulsions from yellow fever patients.

*Disturbances in the Renal Functions.*—In almost all yellow fever cases the urine contains from the 1st day of illness more or less albumin which continues and increases in quantity. On the 2nd or 3rd day granular and epithelial casts appear, being abundant in severe cases. In fatal cases the quantity of urine rapidly decreases the 1st and following days until there may be complete suppression, perhaps anuria, for 24 hours or longer previous to death. The amount seldom exceeds 500 cc. In the non-fatal cases there is a similar diminution of urine during the first 4 or 5 days, but from that time there is a gradual return to the normal output. In many cases the quantity reaches 1,000 cc. on the 8th, 9th, 10th, or 11th day, while in some the secretion may be only 500 cc. or less for many more days. The casts and albumin are less in mild cases and disappear earlier than in the severe cases, in which they may persist for from 2 to 3 weeks. They are present in largest quantity some time during the 2nd week, and as early in rare instances as the 5th or 6th day. The average maximum of albumin is about 5 gm. per liter. In a few instances it went up to 18 gm. In fatal cases the amount of albumin was not very large, being about 1 to 2 gm. per liter, although there were a few instances in which it was somewhat over 5 gm. per liter during 24 hours. In a few apparently severe cases the amount of albumin was comparatively small, 1 to 2 gm.

During the 1st and 2nd weeks of the disease erythrocytes are always found in varying quantities in the urine.

*Jaundice.*—This is one of the most constant manifestations of yellow fever. The intensity of jaundice is, in the majority of cases, in proportion to the severity of the disease. In a very mild case it appears later and disappears sooner than in more severe cases and may be so slight that careful attention alone will reveal its presence. In average non-fatal cases jaundice may be detected on the 3rd day of the disease, first in the scleras and then in the lighter parts of the skin. On the 4th or 5th day the jaundice deepens, and the entire body assumes a light saffron to ocher-yellow hue. In milder cases

the height of jaundice is reached within the 1st week, while in severer cases the color goes on deepening for another week until the appearance of the patient varies from a grayish green to bright yellow. In fatal cases the jaundice may be pronounced or rather slight according to the period of the disease at which death occurred. The longer the patient lives the deeper is the jaundice as a rule. Jaundice becomes more evident after death, owing to the cessation of the blood circulation.

Bile pigments are present in the blood, pericardial, pleural, peritoneal, and cerebrospinal fluid, the adipose tissues, urine, various organs, muscles, glands, the skin, and mucous and serous membranes. The cartilages are also stained. The brains are not noticeably yellow. The urine becomes brownish yellow on the 2nd or 3rd day, and the color becomes deeper as the disease advances until it reaches the color of dark greenish brown or deep brownish yellow. In the bladder of patients dying during the 1st week the urine is of brownish yellow color.

Bile pigments are readily recognizable in the serum or plasma drawn after the 3rd day and give them a deep yellowish brown color in a later stage of the disease.

*Leucocytes.*—As a rule there is a slight hyperleucocytosis on the 1st day of the disease, but the number of leucocytes soon returns to normal, and in a few days a marked leucopenia sets in. There were a few exceptions, however, in which marked hyperleucocytosis was maintained for several days. The differential count showed a high percentage of polymorphonuclear leucocytes.

*Hemorrhages, Vascular Injection, and Herpes.*—Hemorrhagic diathesis is the third cardinal symptom of yellow fever and is never absent from fully developed cases.

During life hemorrhages are manifest in various forms, such as epistaxis, hematemesis, melena, hematuria, gingival hemorrhages, subconjunctival ecchymoses, and in occasional cases subcutaneous ecchymoses and petechiæ. Epistaxis is frequent and often profuse and may be one of the early symptoms. Hematemesis begins usually in the 1st week and becomes severer in the 2nd week. The vomitus is at first yellowish brown, but soon a coffee-ground color to which the term "black vomit" in yellow fever owes its origin. It is

a mixture of mucus, blood clot, and food in a semidigested state. This symptom may be absent in fatal cases, in which the coffee-ground contents may be first found in the stomach at autopsy. In severe but non-fatal cases the black vomit may occur in the 2nd week. Melena is a concomitant of hematemesis and is usually present in all severe cases. Gingival hemorrhages from swollen gums occur in many cases and are sometimes alarmingly persistent and profuse in severe cases. Hematuria is frequently observed, and in fact the urine of yellow fever patients always contains varying quantities of the blood corpuscles when examined under the microscope. Subcutaneous petechial hemorrhages of various sizes, from that of a pin-head to that of a split pea, were observed in several cases (Figs. 1 and 2).

During the 1st week the superficial vascular system seems to be dilated, and the blood-shot appearance of the conjunctival capillaries of the eyes is one of the most constant symptoms of the disease. The patients usually are flushed in the face in the early stage.

Herpes labialis is frequently present during the 1st week of the illness (Fig. 3).

*Pulse.*—Relative brachycardia is another well known characteristic symptom of yellow fever and has been observed in the majority of cases in Guayaquil. In many fatal cases, however, the pulse curve went up above that of the temperature a day or two before death. This disproportion of temperature and pulse is shown in the charts elsewhere recorded in this paper. A patient with a temperature of 39.5°C. may have a pulse of 80, and during the convalescent stage as slow as 45 or 50 beats per minute.

*Nausea and Vomiting.*—Nausea, accompanied by anorexia, is noted from the beginning and is soon followed by vomiting. Vomiting may occur in the beginning, however, or may begin several days later. The character of the black vomitus has already been described. It often appears bilious at first.

*Pains.*—Intense headaches, frontal, orbital, or general, are complained of by all patients during the first 3 or 4 days. Pains in the muscles of the trunk, loins, and calves, and sometimes in the arms and thighs are observed in all cases, being extremely intense in some. They are most marked during the first 3 days; later the patient may

make no reference to them unless questioned, being preoccupied probably by the cephalalgia. Epigastralgia is usually present and is almost intolerable in some severe cases. In mild cases the epigastric region is tender to pressure. Pains in the back in severe cases may be due to the acute nephritis. The liver is palpable, enlarged, and tender to pressure. The spleen is normal. The tongue is coated, with free red tip and edge. The lungs often show slight bronchitis.

*Onset and Course of Yellow Fever in Man.*

In the foregoing paragraphs the clinical features which constitute the disease known as yellow fever have been set forth. By bringing together the symptoms which occur concomitantly during the course of the infection, the reader can picture to himself the appearance of a mild, moderate, severe, or fatal case. A brief resumé is given here, however.

*Mild Infection.*—Onset with severe headache, coated tongue with red tip and edge, suffusion of conjunctivæ, myalgia, anorexia, and nausea, accompanied by a temperature of about 39° or 39.5°C., usually without chill. The patient is rather ill, but still able to go about. There is a moderate hyperleucocytosis which presently may drop to normal or to leucopenia. The pulse is relatively slow. A trace of albuminuria and icterus are present during the next few days. All symptoms rapidly disappear within a week or a little more.

*Moderate Infection.*—All the symptoms just described are present, together with black vomit during the 1st week. The patient is ill enough on the 1st day to go to bed. The presence of albumin and casts in the urine, oliguria, and icterus are rather pronounced and may persist for 10 days or more, when convalescence begins. Gastralgia is present. The liver is palpable and tender.

*Severe Infection.*—All symptoms are much aggravated, and there may be rigor and a fever around 40°C. Epistaxis, hematemesis, hematuria, melena, and gingival hemorrhages follow. Icterus is intense, and the urine diminishes rapidly as the disease advances, and great quantities of albumin, casts, and bile pigments are contained in it. The patient may become delirious at the end of the

1st week. The epigastric pain is severe. Profuse hemorrhages, hypothermia, and exhaustion ensue. By the 12th day the urine begins to increase in volume and continues to do so for subsequent days. With the increased urine the patient begins to improve, and within another week or two all the symptoms except jaundice and albuminuria gradually disappear.

Parotitis and a secondary fever on the 14th to 16th day were observed in certain cases as complications.

*Fatal Infection.*—The onset of the yellow fever that ends in death is the same as that observed in the severe non-fatal cases. Both are very grave from the beginning. Some cases appear to be in a state of exaltation, with brightly flushed face and blood-shot eyes, when brought in on the 2nd day, but the bloody vomit soon begins, and the patient is rapidly seized by an agony of pains. Albumin, casts, and blood cells are present in the urine. On the 3rd or 4th day there may be total anuria. The patient soon becomes delirious, then comatose, and dies in convulsions. There may be a sudden drop in temperature before death or in some instances a sudden rise to near 40°C. When death occurs after the 6th day the temperature is already low, sometimes below 37°C. Jaundice is always present in fatal cases. In some cases the black vomit, or rather hematemesis (still quite bright red) occurs near death or may be found at autopsy.

The foregoing summary is intended only to give a very general impression, the details being recorded in other papers.

### *Autopsy Findings in Yellow Fever in Man.*

This part of the subject is important in completing our knowledge of the disease as it affects man. All the clinical manifestations are only the apparent characteristics of the disease and are explained and extended by the pathological findings.

Postmortem rigor and lividity are pronounced. There is intense jaundice throughout the entire body. The nostrils and mouth may be partly filled with blood clots and the face smeared with blood. The region about the anus is often stained with melena. Uterine hemorrhages were observed in young women. The skin, subcutaneous tissues, and muscles are yellow.

*Lungs.*—Often edematous; show marked hypostasis; crepitant. In every case there were ecchymotic hemorrhages, variable in size and distribution. The size varies from a few millimeters square to the size of a split pea, and is sometimes as large as a pigeon's egg. The hemorrhagic foci are sharply defined in some and diffuse in others. If recent they are vivid red, if several days old bluish red. On section they are seen to extend deep into the substance of the lungs, some into the interior. They are discrete and multiple, and the number varies in different cases and in the two lungs.

*Heart.*—Often shows hemorrhagic foci in the pericardium, which contains icteric fluid and is often dilated on the right side and in diastolic condition. The myocardium is grayish yellow, brittle, and cloudy. Numerous punctiform ecchymoses are frequently found on the surface. The endocardium is clear, with occasional punctiform hemorrhages along the papillary muscles. The valves are intact.

*Liver.*—Hyperemic and often somewhat enlarged. The color varies, yellowish brownish red, ocher-yellow, saffron-yellow, light greenish yellow, brownish yellow. The whole may be of uniform color or shaded, blended, or minutely mottled. The yellower or greener color indicates a more advanced stage of degeneration, in which the parenchyma is brittle and tears easily when handled with forceps. In a brownish red liver the tissue still retains much of its normal consistency. Minute ecchymotic spots are sometimes visible on the surface.

*Gall Bladder.*—Usually full of deep greenish yellow bile. There may be multiple ecchymoses in the wall.

*Kidneys.*—Enlarged, highly hyperemic, and reddish yellow in color. Varying number of ecchymoses, some punctiform and some as large as a bean, may be present in the capsule, which strips easily. In a few instances punctiform hemorrhages were found in the cortex. On section hyperemia is noticed along the junction of the cortex and medullary portion. The cortex is broader and shows general swelling and cloudiness. The medulla is succulent and icteric. The renal pelvis may be free, but not infrequently it contains a blood clot, or numerous punctiform hemorrhages are seen irregularly situated. The kidneys, therefore, show acute parenchymatous inflammation.

*Suprarenal Glands.*—Appear to be congested and more friable than normally. In one instance an extensive hemorrhage was found in the perinephritic adipose tissue.

*Gastrointestinal System.*—The stomach usually contains a viscid semifluid of coffee-ground appearance, known as the black vomit or *vomito negro.* In some instances freshly extravasated blood gives the gastric contents a dark reddish hue, or they may be dark green in color with black particles. The serosa is free from any ecchymoses, but the mucosa is intensely injected with more or less numerous ecchymoses, particularly near the cardia.[3] The small intestines as well as the colon are similarly affected and contain a tarry fluid (melena).

*Bladder.*—Except for occasional ecchymoses on the serosa nothing special has been observed. The bladder is often full.

*Uterus and Ovaries.*—Intense injection of the endometrium and sometimes hemorrhage in the uterus. The ovaries are somewhat injected. In one instance there was hemorrhage of both ovaries.

*Testicles.*—No change found.

*Spleen.*—Apparently normal.

*Lymphatic Glands.*—Swelling and hemorrhages were observed in the bronchial, mesenteric, and other lymphatic glands. This condition, as well as the constant ecchymoses in the lungs, seems to have received little attention from previous investigators.

*Serous Membranes.*—Pleuræ, peritoneum, and omentum are usually free from hemorrhages.

*Skin and Mucous Membranes.*—Occasional petechial hemorrhages were observed.

*Nervous System.*—The central nervous system is macroscopically unchanged. The cerebrospinal fluid is icteric and the brain edematous. The membranes are injected.

### *Histological Findings.*

*Lungs* (*Figs. 4 to 7*).—In the majority of instances areas of hemorrhage of varying extent are found and also small foci of infiltration with polymorphonuclear leucocytes. The alveoli are filled with

[3] This has been constantly observed by Dr. Pareja in his long experience.

polymorphonuclear and large endothelial cells and red blood corpuscles. The thrombi in some vessels are similar to those seen in infarctions.

*Liver* (*Figs. 8 to 11*).—The liver is necrotic in the large proportion of cases, and the parenchyma is full of extravasated blood. Only around the vessels are the living cells found, better preserved around the central vessel than around the portal canal. A moderate amount of pigment is sometimes found within the bile capillaries. The blood is distributed throughout the necrotic areas and not always confined to the blood vessels. Mitotic figures are met with among the liver cells. Most liver cells are vacuolated, and some are distended with vacuoles. Hemorrhagic areas are found. Numerous red-staining cells mingle with those less degenerated. There are a few granules in the liver cells but no pigment. In the region of the portal canal small foci of lymphoid and plasma cell infiltration are encountered.

*Kidneys* (*Figs. 12 to 15*).—The convoluted tubules are somewhat dilated with granular excretion. There are many deeply stained hyaline and granular casts. The epithelium of the convoluted tubules shows granular and somewhat vacuolated cytoplasm. The glomeruli are moderately or considerably injected; there is rather marked injection of the medulla with several hemorrhagic areas.

*Stomach.*—A few minute foci of infiltration with polymorphonuclear leucocytes; in certain areas a more diffuse infiltration of lymphoid and plasma cells (some bacilli). The superficial portion of the mucosa is markedly congested. The mucosa is somewhat injected in places.

*Large and Small Intestine.*—Injection and occasional hemorrhages.

*Heart.*—The muscle fibers show one or more vacuoles situated in the central portion, suggestive of fat. Certain fibers appear somewhat swollen. The nuclei are large and vesicular.

*Spleen.*—Numerous large phagocytes containing red cells. Follicles atrophied in one case; in another much blood in the pulp. A large number of phagocytic cells appear in certain areas. These contain red cells and red-staining granules.

*Lymph Nodes.*—(1) Some degeneration and phagocytosis centrally situated in the follicles. (2) Peripheral sinuses filled to a large extent

with polymorphonuclear leucocytes and a small number of phagocytic cells. The lymph follicles show small areas of degeneration centrally situated.

*Pancreas.*—Certain of the gland alveoli show small groups of degenerated cells. Sometimes there are no lesions.

*Adrenals.*—There is a marked degree of parenchymatous degeneration, affecting chiefly the medulla, and considerable congestion. In some cases the adrenals are intact.

*Nervous System.*—Nothing abnormal.

## SUMMARY.

The clinical and pathological features of the yellow fever prevalent in Guayaquil conform with those described by other investigators of this disease as it has occurred elsewhere, both epidemically and endemically.

## EXPLANATION OF PLATES.

### PLATE 31.

FIG. 1. Patient Ch. The photograph, which was taken on the 5th day of the disease, shows large and small areas of subcutaneous hemorrhage on chest and arms, also on the temple. The blotch on the left breast was caused by the application by the patient of mustard plaster. The patient died on the 7th day.

FIG. 2. A patient who was severely ill but finally recovered. There were extensive subcutaneous hemorrhages all over the body of a mottled appearance but with no actual circumscribed spots.

FIG. 3. Patient Co. The photograph, which was taken on the 3rd day of the disease, shows herpes labialis, particularly of the lower lip. The patient died on the 4th day.

### PLATE 32.

FIGS. 4 to 7. Sections of lung from four different cases of yellow fever, fixed with Zenker's fluid and stained with eosin and methylene blue. Hemorrhagic areas, varying in extent, can be seen in all these lesions. They are well defined with respect to the normal tissue (Fig. 4). There is a varying degree of edema. Fig. 4 is from a patient dying on the 6th day, Fig. 5 from a patient dying on the 5th day, Fig. 6 from a patient dying on the 8th day, and Fig. 7 from a patient dying on the 7th day. × 150.

## Plate 33.

Figs. 8 to 11. Sections of liver from cases of yellow fever, fixed with Zenker's fluid and stained with eosin and methylene blue. The general character of the lesion consists in necrosis and vacuolization of the liver cells. Some groups of cells are completely disintegrated. The necrotic areas are occupied by debris and hemorrhage, particularly marked in Figs. 10 and 11. Fig. 8 is from a patient dying on the 6th day, Fig. 9 from a patient dying on the 5th day, Fig. 10 from a patient dying on the 8th day, and Fig. 11 from a patient dying on the 7th day. × 150.

## Plate 34.

Figs. 12 to 15. Sections of kidney from cases of yellow fever, fixed with Zenker's fluid and stained with eosin and methylene blue. The general character of the lesion is the same in all these sections: swelling and degeneration of the renal epithelia in the tubules with varying degrees of hemorrhage into the connective tissue. The glomeruli are highly congested; some of the epithelia of the tubules are vacuolated and desquamated. The lumina of the tubules are filled with granular casts. Fig. 12 is from a patient dying on the 6th day, Fig. 13 from a patient dying on the 5th day, Fig. 14 from a patient dying on the 8th day, and Fig. 15 from a patient dying on the 7th day. × 150.

# ETIOLOGY OF YELLOW FEVER.

## II. Transmission Experiments on Yellow Fever.

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plate 35.

(Received for publication, March 27, 1919.)

For the purpose of transmitting yellow fever to animals experiments were made to reproduce the disease in whatever kind of animal was available by inoculation with the blood from patients admitted to the Yellow Fever Hospital in Guayaquil during my stay there. The blood was drawn from the median basilic vein of the patient at various stages of the disease, mainly during the 1st week, with a sterile Luer syringe and injected before coagulation into the peritoneal cavity (in mammals) or into the pectoral muscles (in birds). 10 cc. of blood were usually taken and distributed among from one to four animals in each instance. When more than one kind of animal was used for one patient correspondingly larger quantities of blood had to be drawn. The following animals were employed: ringtail monkey, rabbit, guinea pig, cat, dog, donkey, guatusa,[1] comadreja,[2] and ozo-melero[3] among the mammalians; pigeon, paloma de tierra,[4] bluebird, mantas,[5] blackbird, parrakeet,[6] reedbird, blancos,[7] and diostede[8] among the birds.

[1] *Dasyprocta aguti* (animal akin to rabbit).
[2] *Bassaris bassaricyon* (?) (resembling an opossum).
[3] *Cholopus didactylus* (two-toed sloth).
[4] Family of Peristerinæ (ground-dove).
[5] Siskin (probably).
[6] Family of Bolborhynchus.
[7] Similar to a magpie, but white.
[8] Genus *Pteroglossus*.

All the animals inoculated were kept under observation at least 1 month before being discarded as negative, even when no symptoms were observed. An animal which showed a rise of temperature after a period of several days was closely watched, and when more than one of the animals of the series receiving the same blood became febrile one was killed for examination and further passage made in the same species of animal. As will be seen later (Case 2) such a step was essential with some specimens of yellow fever material, as by this means alone was it possible to reproduce an experimental condition resembling yellow fever on the third animal passage.

None of the birds showed any definite symptoms when first inoculated with the blood of yellow fever patients, but upon subsequent injection of the organ emulsion of a guinea pig with the experimental yellow fever[9] parrakeets, mantas, blackbirds, reedbirds, and paloma de tierras died within 24 to 72 hours. There were no lesions except for hyperemia of the visceral organs. The pigeons, bluebirds, and toucans showed no definite reaction. This rather rapid death following the second inoculation may have been due to an anaphylactic phenomenon. Rabbits and guatusas showed a temperature of 40–41°C. on the 4th to 5th day after inoculation but became normal after 48 hours. There were no other symptoms, and the animals remained well during the period of observation (1 month). The ringtail monkeys showed a similar but more prolonged fever reaction lasting several days. The animals appeared depressed after 48 hours and refused to take food. The conjunctivæ were injected for several days, but there was no jaundice. Unfortunately the number of experiments with this animal was limited to five because of the difficulty in securing them in sufficient numbers. Donkeys showed no reaction to the injection of yellow fever blood in large quantities.

Apparently none of the animals enumerated above possesses a sufficient degree of susceptibility to enable the yellow fever virus to multiply and reproduce the symptoms and lesions known as yellow fever in man. It is possible that some of them had an extremely mild or atypical form of the infection.

[9] For the sake of brevity the condition induced by inoculation will be termed "experimental yellow fever." Further evidence bearing on the validity of this term will be presented in successive papers of the series.

Since guinea pigs could be secured more easily, and in view of the success of Inada and Ido[10] with this species in the study of infectious jaundice, they were extensively used during the present study. Guinea pigs are native to Ecuador and are found wild in the mountainous regions.[11] All except 60 of the guinea pigs which were used in these experiments (which were taken from New York) were reared in the mountains and shipped to the Yellow Fever Hospital. They stand captivity well for several months.

*Experiments with Guinea Pigs.*

*Blood and Organ Emulsions.*—Of 74 guinea pigs inoculated with the specimens of blood from 27 cases of yellow fever, 8, representing 6 cases, came down with the symptoms resembling human yellow fever. In one instance, however, the reproduction of the disease required three successive inoculations in guinea pigs. In this instance one of the animals showed a rise of temperature on the 5th day and was killed for examination and transfer on the 6th day. The liver was degenerated, and the kidneys were highly congested. A few hemorrhagic spots were found in the lungs. In a further passage of the organs to two new guinea pigs there was a similar febrile reaction with ecchymoses in the lungs but only a trace of icterus. On transfer of the second passage material typical hemorrhages, icterus, and albuminuria were induced. With the materials from only one of several autopsies was a positive transmission obtained. The details of the transmission experiments follow.

The first positive transmission was obtained in the case of Patient A.

*Case 1* (*Text-Fig. 1, a*).—A. A., female, age 17 years; servant, native of Latacunga (9,055 feet above sea level). Had come to Guayaquil 3 months previously.

Onset, July 14, 1918. Headache, chills, fever, and severe pains throughout the body. July 15. Vomited chocolate-colored matter and felt pains in epi-

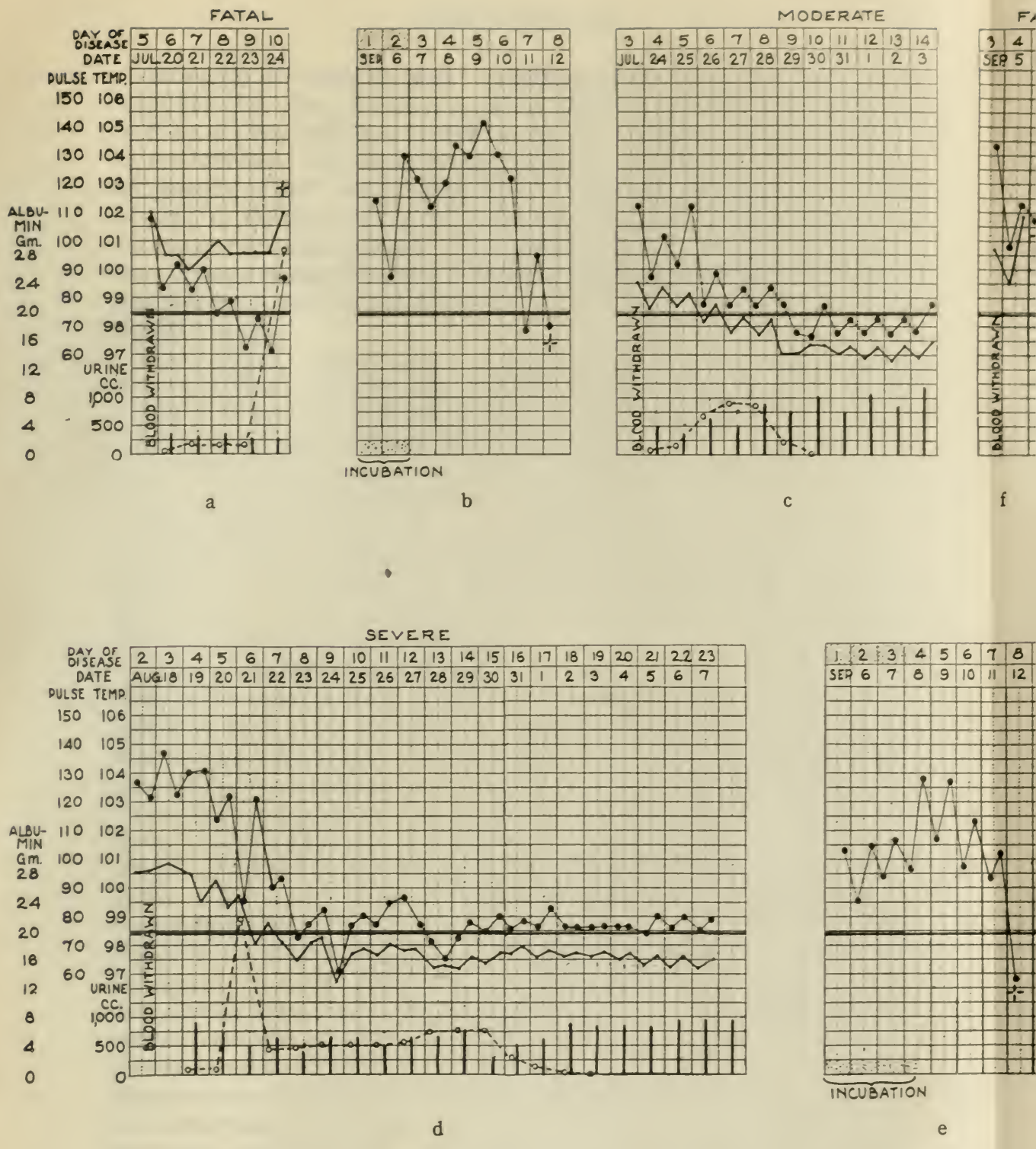
[10] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

[11] Guinea pigs are in the market for food in Ecuador, but the municipality of Guayaquil has forbidden their being kept in the city on account of the possibility of spreading bubonic plague.

gastric region. July 19. Admitted to hospital. Was very sick and vomited dark bloody matter. Conjunctivæ intensely injected and distinctly jaundiced. Liver tender but not palpable. General jaundice of the skin. Blood drawn from median basilic vein into citrate solution and inoculated into guinea pigs immediately and also after 3 days cultivation at 25°C. July 20. Patient felt better, but gums began to bleed. Abdominal pains not marked. July 22. Condition worse. Patient very ill, vomited constantly ("coffee-grounds vomitus"). Jaundice very marked. Liver tender and swollen. Tumefaction of thighs because of menstruation. July 23. Delirium and insomnia with intense epigastric pains. July 24, 9 p.m. Died.

*Autopsy.*—Performed 11 hours post mortem. Well nourished young woman, with well marked rigor mortis and lividity. Body cyanotic and mottled. Pronounced general jaundice. Mouth and nostrils contained dark, partly dried blood clots, and face was badly smeared with dried blood. Similar blood specks were found on the hands and the genital regions, the skin of the thighs being extensively excoriated owing to hemorrhages from the uterus. No petechiæ or subcutaneous ecchymoses present. Lungs edematous. Pronounced hypostatic congestion of the dependent parts. Several ecchymoses of pea size present. Pleura free, containing moderate amount of deeply jaundiced clear fluid. On section the hemorrhagic spots of the lungs were seen to extend many centimeters into the parenchyma; some occult foci of similar ecchymoses in the interior. Some old and some new foci of tuberculosis in the upper lobes. Pericardium apparently free from any changes; scanty icteric fluid in the cavity. Heart normal in size; in diastolic state, containing fluid blood in right heart. Few minute ecchymoses on the surface. Muscles pale, flabby, and brittle. Endocardium and valves normal except for a few petechial hemorrhages on the papillary muscles. Liver pale ocher-yellow in color, fatty, and streaked with congested areas. Color on section uniformly yellow; parenchyma friable. Stomach distended with gas, containing blackish bloody mucous material; mucosa hyperemic, especially a few minute hemorrhagic spots near the cardia. Intestines filled with dark tarry contents; mucosa hyperemic. Kidneys swollen and hyperemic, capsules non-adherent; on section numerous ecchymoses in the pelvis; increase of the cortical layer and cloudy swelling. Deeply jaundiced. Suprarenal glands hyperemic. Spleen and pancreas apparently normal. Ovaries congested and uterus hyperemic, containing extravasated blood in the cavity. Small amount of deeply yellowish urine in bladder.

*Animal Inoculation.*—The blood, drawn on the 5th day of disease, was used in two different ways. One portion was immediately inoculated into the peritoneal cavity of two guinea pigs, and the other was put into a mixture of Ringer solution 3 parts and human serum 1 part with 0.3 per cent neutral agar, then well mixed and covered with a layer of paraffin oil. This semisolid mixture was placed in a thermostat at room temperature for 3 days (20–25°C.) and then injected into two guinea pigs intraperitoneally. The amounts of the original blood injected into each guinea pig were from 2 to 3 cc. The protocols follow.




TEXT-FIG. 1, *a* to *f*. Cases of yellow fever in which positive transmissions to guinea pigs were obtaine (*a*) Case 1. Age 17 years. (*b*) Guinea Pig 129 A. Strain, Case 1. (*c*) Case 2. Age 23 years. (*d*) Ca 3. Age 20 years. (*e*) Guinea Pig 353 C. Strain, Case 3. (*f*) Case 4. Age 15 years.





*Guinea Pig 50.*—July 19, 1918. Inoculated with 3 cc. of the fresh blood intraperitoneally. The temperature rose to 39.6°C. on the 6th day, dropped to 38.5° on the 7th, again rose to 39.8° on the 8th, was 40° on the 9th, and 39.7° on the 10th day. The animal became less active, and on the 11th day it had a temperature of 39.1°, which continued for 3 days and then returned to normal (38–38.5°). On the 11th day the scleras appeared icteric, and the capillaries of the conjunctiva much suffused. The animal recovered completely in 15 days. On a subsequent injection (Aug. 10, 1918) with material taken from other guinea pigs inoculated with the enriched blood of the same patient this animal failed entirely to react.

*Guinea Pig 51.*—July 19, 1918. Inoculated intraperitoneally with 2 cc. of the same lot of blood. The temperature was 39.9°C. on the 9th day, 40.5° on the 10th, and 39.2° on the 11th, returning gradually to normal (38.5–39°). There was a suspicion of jaundice on the scleras on the 12th day which disappeared in a few days. This animal remained well and was subsequently (Aug. 10, 1918) tested against material taken from this case. It failed to react.

*Guinea Pig 80.*—July 22, 1918. 4 cc. of the mixture of the citrate blood and the culture medium, which had been incubated for 3 days, were injected intraperitoneally into the guinea pig. The temperature was 40°C. on the 5th day, 40.1° on the 6th, and 38° on the 7th, when the animal became distinctly jaundiced. Death occurred the same day (July 29).

*Autopsy.*—Marked general jaundice. No definite subcutaneous ecchymoses. Dried blood specks around the nostrils. Mucous membranes very yellow. Lungs congested; showed few disseminated ecchymotic spots which on section were seen to extend deeper into the parenchyma. The size varied from a minute point to that of a split pea, and the color from bright red to dark bluish red. The contour of these areas was usually sharply demarcated from the normal tissue. Lungs edematous and the lower lobes highly hypostatic. Pleuræ not affected. Right heart appeared dilated and contained semicoagulated cyanotic blood. A few punctiform hemorrhages on the anterior surface. Muscles friable. Liver yellowish brown in color and mottled with areas of congestion. The markings of the lobules were prominent. Consistency firmer and less succulent than the normal. Stomach distended with undigested food partly tinted with the dark blood which escaped from the hemorrhagic spots on the highly congested mucosa, especially marked near the cardia. Intestines highly hyperemic and showed numerous spots of hemorrhage along the mucosa. Contents bloody. Kidneys intensely hyperemic and more or less swollen, showing a few punctiform hemorrhages in the cortex. On section the pelvis was filled with blood and there were some ecchymoses. The cortex was broad and red, and the medulla succulent and cloudy. Adrenals hyperemic and swollen. Spleen, pancreas, bladder, and testes apparently unaffected. Along the abdominal wall the muscles were congested and showed some disseminated ecchymoses.

*Microscopic Examination.*—The blood was apparently free from any microorganism when examined under the dark-field microscope. Emulsions were

prepared from the liver, kidney, and adrenals for examination by dark-field and also for further passage. A very few actively motile organisms belonging to the genus *Leptospira* were demonstrated in the liver and kidney emulsions but not in the adrenal.

*Passage.*—Two guinea pigs were immediately inoculated with a mixture of the emulsions of the liver and kidney from Guinea Pig 80 and a further generation of this strain was obtained in the two following guinea pigs.

*Guinea Pig 80 A.*—July 29, 1918. The organ emulsion (2 cc.) was inoculated intraperitoneally. The animal showed a temperature of 41.3°C. on the 4th day, 40.6° on the 5th, and 38.7° on the 6th, when the color of the skin and mucosa became intensely icteric. Death occurred at 3 p.m. on Aug. 3 while the animal was being placed in a mosquito cage with the stegomyias.

*Autopsy.*—The general appearance was the same as that of Guinea Pig 80. The urine contained bile pigment, albumin, and abundant casts. The leptospira could not be demonstrated either in the blood, liver, or kidney.

*Guinea Pig 80 B.*—July 29, 1918. The emulsion was applied to the scarified surface of the skin. The highest temperature (morning) was 39.9°C. on the 7th day. It went down to 38.8° on the 9th day, when the animal became very yellow. The animal died on the 10th day (Aug. 7).

*Autopsy.*—About the same as in the foregoing experiment except for a higher degree of degeneration of the liver, which was ocher-yellow, exactly as in some human cases. No leptospiras were demonstrated by the dark-field microscope, but a further successful transfer was made.

*Guinea Pig 81.*—July 22, 1918. 5 cc. of the enriched blood of Patient A. were injected intraperitoneally. The temperature was 40.3°C. on the 6th day, 40° on the 7th day, 39.9° on the 8th, and 38.7° on the 9th day, when jaundice became noticeable. The animal was found dead on July 31.

*Autopsy.*—Lesions similar to those already described in Guinea Pig 80. The stomach was filled with a semifluid blackish gray matter (digested blood). Dark-field examination of the blood, liver, and kidney failed to demonstrate any organism. Transfers were made to six guinea pigs. The protocols are given below.

*Passage.*—July 31, 1918. Six guinea pigs were inoculated with the emulsions of the kidney and liver, intraperitoneally and also percutaneously after depilation.

*Guinea Pig 124.*—July 31, 1918. Kidney emulsion (1 cc.) intraperitoneally. The animal had a temperature of 40.1°C. on the 4th, 40.5° on the 5th, 39.6° on the 6th, and 39.9° on the 7th day. It continued to show high temperature for 14 days, but jaundice was not present at any time. It was discarded after 30 days (Aug. 30).

*Guinea Pig 125.*—The same material was used as in the foregoing experiment. The animal had a temperature of 40°C. on the 4th and 40.1° on the 5th day. After that it was 39.5° or less until the 11th day, when the animal became quite icteric. It collapsed while being used for infecting stegomyias in a cage (Aug. 10,

4 p.m.), and was killed for examination of blood, liver, and kidney. Considerable numbers of leptospiras were found in the blood and organs. Further transfers were made with success. A culture was obtained from the heart's blood, but within a week a fungus contamination destroyed it.

*Passage. Guinea Pig 126.*—July 31, 1918. The liver emulsion (1 cc.) of Guinea Pig 81 was inoculated into the peritoneal cavity. The temperature went up to 40°C. on the 5th, 6th, and 9th days; the animal showed definite jaundice on the 10th day and died on the 11th. The chief symptoms were all very marked.

*Guinea Pig 127.*—This was a duplicate of the foregoing experiment. The animal died of a secondary infection within 3 days.

*Guinea Pig 128.*—Percutaneous inoculation with a mixture of the liver and kidney emulsions of Guinea Pig 81. The animal had a temperature of 40°C. during the 10th, 11th, and 12th days, but it had normally registered as high as 39.5°. There was a trace of jaundice on the 13th day, which soon disappeared. The animal remained well for 31 days and was discarded as negative.

*Guinea Pig 129.*—This was a duplicate of the foregoing experiment. Temperature 40.3°C. on the 7th and 40.1° on the 8th day, when a suspicion of jaundice appeared. It was killed on the 8th day in order to ascertain the lesions and obtain material for culture and transfers.

*Autopsy.*—Jaundice too slight to be definitely recognized. The lungs, however, showed the beginning of ecchymotic spots, and the liver and kidneys were highly congested. Dark-field examination failed to reveal any organism. Transfers were made to two guinea pigs on the same day (Aug. 7). One of them died with mild but typical lesions, while the other completely recovered after having shown a temperature of 40° and 40.4°C. on the 7th and 8th days.

Text-fig. 1, *b* is the chart of Guinea Pig 129 A, which succumbed to the intraperitoneal inoculation of 0.5 cc. of a culture of this strain (Aug. 27–Sept. 5, 1918), with typical symptoms and lesions. The leptospira was demonstrated in the liver.

The foregoing experiments are of great interest as they indicate that the microorganism which was responsible for the disease and subsequent death of this patient was apparently successfully transmitted to the guinea pig. The failure of the unmodified blood to reproduce the disease in guinea pigs as contrasted with successful transmission by means of the same blood after a temporary enriching of the virus *in vitro* may be explained by the fact (as subsequent experiments concerning the properties of the serum of yellow fever patients and the properties of the organism indicate) that the inoculation of the fresh blood carried with it a certain amount of antibodies antagonistic to the development in the body of the guinea pig, while this

property is certain to be considerably modified when incubated in the culture medium employed. Moreover, as the organism is capable of multiplying outside the body at room temperature there is temporary enrichment of the organisms before inoculation into the guinea pig.

It is also shown in this experiment that the microorganism is variable in virulence, being able to produce a fatal infection in some, an abortive infection in others, and in still others no infection at all. The organism is capable of entering the body of the guinea pig in some instances by the smearing of the infective material. That there is an abortive form of the infection is shown by the immunity that it confers upon the guinea pigs which survived such an infection. The infective agent may be passed from one guinea pig to another by timely transmission.

A leptospira has been found to be associated with this case, but its demonstration by means of the dark-field microscope is not always successful; it may or may not appear in a subsequent passage. That this organism, notwithstanding the difficulty of demonstrating it, is etiologically related to the disease known as yellow fever in Guayaquil is made highly probable by subsequent experiments.

Fig. 1 shows the organism in the blood of a guinea pig inoculated with the culture of this strain.

The second positive transmission was obtained in the case of Patient P.

*Case 2* (*Text-Fig. 1, c*).—C. P., male, age 23 years; tailor, robust, a native of Cuenca (highland). Had been in Guayaquil a month.

Onset July 20, 1918. Felt fever without chills; severe headache, photophobia, muscular pains throughout body, cramps in legs. No nausea. July 23. Admitted to hospital. Marked injection of the conjunctiva; slight jaundice; much albumin in the urine. Liver small but tender (Dr. Elliott). Temperature 38.9°C. Blood was drawn from the median basilic vein and injected into two guinea pigs (see protocols). July 24. Epistaxis in the morning. July 25. Nauseated easily; diarrhea. Little urine since yesterday. July 26. Jaundice advanced. Albuminuria +++. July 31. Improving. Temperature normal. Aug. 2. Trace of jaundice. No albumin in the urine. Aug. 3. Discharged.

*Animal Inoculation. Guinea Pig 88.*—July 23, 1918. Intraperitoneal injection of 4 cc. of the blood of Patient P. (4th day of disease). Temperature rose to 39°C. on the 4th and 40° on the 5th day. The animal was killed on the 6th day for further passages into two guinea pigs.

*Autopsy.*—Lungs showed a few hemorrhagic spots. Liver congested and somewhat yellowish (fatty). Kidneys congested and cloudy, showing swelling on section. Gastrointestinal system hyperemic but showing no hemorrhages. Spleen normal.

*First Passage. Guinea Pig 88 A.*—July 28, 1918. Received 1 cc. of blood of Guinea Pig 88 intraperitoneally. The temperature rose to 39.9°C. on the 7th day; killed on the 10th day for examination and transfer.

*Autopsy.*—Slight icterus. The lungs were spotted with a few ecchymoses. Liver congested and perhaps fatty. Kidneys congested and showed cloudy swelling on section. Intestine hyperemic and showed scattered hemorrhagic foci.

The liver and kidneys were emulsified and used for inoculating two normal guinea pigs (see second passage). Leptospiras not found.

*Guinea Pig 88 B.*—Duplicate of No. 88 A. This animal showed a slight rise of temperature in 4 days, but during observation for further evolution of the reaction it became normal and was discarded on Aug. 22.

*Second Passage. Guinea Pig 89 A.*—Aug. 6, 1918. 1 cc. of the mixed emulsion of the liver and kidney of Guinea Pig 88 A was given intraperitoneally. The temperature went up to 40.9°C. on the 3rd, 39.6° on the 4th, and 40° on the 5th day, but fell to 38.6° on the 6th day. Epistaxis and icterus. The animal was found dead on the 7th day.

*Autopsy.*—Extremely jaundiced. Lungs hemorrhagic; liver yellowish; kidneys congested, with some ecchymoses. Stomach and intestines contained blackish matter and numerous hemorrhagic areas were present on the mucosa. Leptospiras were demonstrated in the liver and kidney but not in the blood. Transfers to two normal guinea pigs were made, but this strain was lost because of intercurrent infection.

*Guinea Pig 89 B.*—Duplicate of No. 89 A. This animal showed less fever than usual in the 1st week but 40°C. on the 10th day. It was kept under observation until Aug. 22, but nothing further developed.

*Guinea Pig 89.*—This was one of the two guinea pigs inoculated with the blood of Patient P. on July 23. This animal showed a temperature of 39.3°C. on the 4th, 39.9° on the 5th, 39.2° on the 6th, 38.9° on the 7th, 39.4° on the 8th, and 39° on the 9th day. It was found dead on the 10th day.

*Autopsy.*—Congestion of the lungs, liver, and kidneys, but no icterus. No transfer was made.

The third positive transmission was obtained with the blood of Patient G.

*Case 3* (*Text-Fig. 1, d*).—M. G., male, age 20 years; servant, robust, a native of Ambato (8,435 feet above sea level); had been in Guayaquil 2 months.

Onset Aug. 16, 1918, 10 a.m. General pain and aching; headache; fever without rigor; no nausea, vomiting, or bleeding. Aug. 17. Admitted to hospital. Eyes injected, face flushed; much prostrated. Pains in the head and limbs were

intense. Albuminuria slight; no casts. Temperature 39.9°C. Blood taken for transmission experiments. Aug. 18. Albuminuria increasing; temperature 40.3°. Condition worse. Total leucocytes 9,200 (Dr. Elliott). Aug. 19. Albuminuria increasing and numerous casts in the urine. Temperature still 40°C. Aug. 21. Delirious; otherwise condition the same. Aug. 22. No urine obtained; gums bleeding; jaundice definite; remission in fever. Aug. 23. Decided improvement; temperature going down. Aug. 26. Beginning hematuria; increased icterus. Aug. 29. Continued hematuria; great prostration. Aug. 30. Hematuria diminished; general improvement. Sept. 1. Continued improvement; cessation of hematuria. Sept. 2. Continued improvement; intense jaundice. Sept. 3. Slight pain in bladder. Sept. 7. Discharged.

*Animal Inoculation. Guinea Pig 351.*—Aug. 17, 1918. 3 cc. of the blood of Patient G. (2nd day of disease) were given intraperitoneally. The animal was kept under observation until Sept. 7 but remained without any noticeable reaction except that its temperature rose to 40.1°C. on the 18th day. Discarded as negative.

*Guinea Pig 352.*—Aug. 17, 1918. 5 cc. of the same specimen of blood were given. Temperature 40°C. on the 7th day, 39.8° on the 8th, and 39.6° on the 9th, but later returned to normal (38.6° for this animal). Tested for immunity on Sept. 7 against the A. strain and found to be resistant.

*Guinea Pig 353.*—Aug. 17, 1918. 6 cc. of the blood from Patient G. were inoculated intraperitoneally. The temperature rose to 40°C. on the 7th and 39.6° on the 8th day, but dropped to 38° on the 9th day. The animal was markedly icteric and was killed for examination of the blood and organs as well as for transfer and cultivation of the virus.

*Autopsy.*—The lungs showed a moderate number of ecchymoses. Liver light yellowish brown in color. Kidneys showed acute parenchymatous nephritis; no ecchymoses. Stomach contained a few hemorrhagic foci near the cardia with blood-stained contents. Intestines hyperemic but with few hemorrhages. Intense jaundice everywhere. Spleen normal in appearance. After a long search a few leptospiras were discovered, but the number was so extremely small that preliminary examination revealed none. Transfers were made successfully to two normal guinea pigs, and cultures were also obtained. The organism was demonstrated in the blood of guinea pigs inoculated in later experiments with this strain (Fig. 2).

*First Passage. Guinea Pig 353 A.*—Aug. 26, 1918. Inoculated with 1 cc. of the blood of Guinea Pig 353. Except for a slight rise in temperature nothing happened during the period of observation (up to Sept. 10).

*Guinea Pig 353 B.*—Duplicate of the foregoing experiment. This animal came down with typical symptoms and lesions on Sept. 5, 1918; that is, on the 10th day after the transfer.

*Second Passage. Guinea Pig 353 C.*—Sept. 5, 1918. Inoculated with the emulsion of liver of Guinea Pig 353 B. The course of the infection was similar to that of the positive instance, with the temperature as shown in Text-fig. 1, *c*.

The fourth positive transmission was obtained with the blood of Patient Co. Successful transmission was also made with the liver emulsion of this patient, as described below.

*Case 4* (*Text-Fig. 1, f*).—J. Co., male, age 15 years; servant, native of Cuenca (highland). Had been in Guayaquil a month and a half.

Onset Sept. 2, 1918. Headache and pains in waist and legs; fever. Sept. 4. Admitted to hospital. Face flushed; conjunctiva injected and slightly jaundiced; gums reddish and swollen; tongue coated, with red tip and edge. Herpes labialis present.[12] No epigastric pain. Blood drawn for inoculation. Sept. 5. Passed no urine, but appeared calm. Increased jaundice. In the afternoon became excitable, anxious, complained occasionally of dyspnea. Melena. Sept. 6. Coma began previous night. Black vomit. Complete anuria. Death occurred at 9 a.m.

*Autopsy.*—A partial autopsy within 1 hour after death. Liver highly degenerated and yellow. In the emulsion a few leptospiras were found. Kidney congested, cloudy swelling. No leptospiras.

*Animal Inoculation. Guinea Pig 435.*—Sept. 4, 1918. Intraperitoneal injection of 3 cc. of the blood of Patient Co. (3rd day of disease). The animal showed a temperature of 39°C. on the 8th, 40° on the 9th, 39.9° on the 10th, and 39° on the 11th day, but subsequently a normal temperature. It was discarded as negative after 25 days.

*Guinea Pig 436.*—Duplicate of the foregoing experiment. 5 cc. of the blood injected. The temperature rose to 39.9°C. on the 9th and 40.1° on the 10th day, coming down to 38.2° on the 12th day. The animal showed distinct icterus on the 11th and 12th days and died during the night of the latter.

*Autopsy.*—General jaundice. Hemorrhages in the lungs and gastrointestinal tract. Liver brownish yellow. Kidney highly congested; showed cloudy swelling on section. Other organs normal in appearance. Leptospira found under the dark-field microscope and in stained slides (Fig. 3).

The fifth positive transmission was obtained with the emulsion of the liver of the same patient, who died on the 5th day. The material was taken from the body within 1 hour, and the emulsion made with Ringer's solution was inoculated intraperitoneally into four guinea pigs, the quantity for each being 1 cc. A few distorted immobile leptospiras were demonstrated in this emulsion under the dark-field microscope.

*Guinea Pigs 444 and 446.*—Sept. 6, 1918. Received 1 cc. of the liver emulsion from Patient Co. Died on the night of the following day.

[12] See Fig. 3, Paper I.

*Guinea Pig 447.*—Duplicate of the foregoing experiment. This animal never showed a reaction and died from an intercurrent infection after 22 days.

*Guinea Pig 445.*—Duplicate of the foregoing experiment. The animal had a temperature slightly above 39°C. for 6 days which rose to 39.9° on Sept. 13 and to 39.7° on the following day. Sept. 15. The animal was distinctly icteric and was killed for examination and transfer.

*Autopsy.*—Typical lesions in the lungs, stomach, liver, and kidneys. The blood, as well as the liver and kidney, showed a few leptospiras.

*Passage.*—From this animal the strain has been successfully passed through many generations. A culture also was obtained from a later passage.

The sixth positive transmission was obtained with the blood of Patient Ch., drawn from the median basilic vein on the 5th day of the disease and 48 hours previous to his death. In this instance 2 cc. of the fresh blood were directly inoculated into the peritoneal cavity of each of the two guinea pigs.

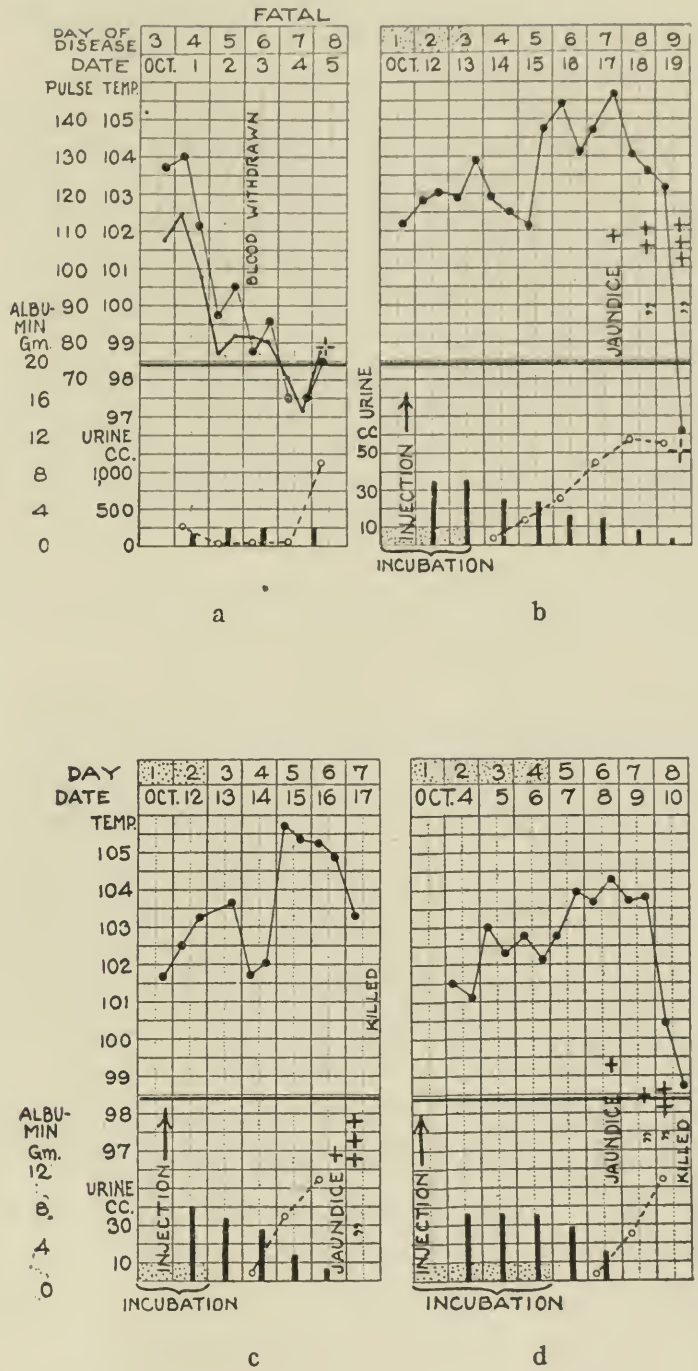
*Case 5* (*Text-Fig. 2, a*).—E. Ch., male, age 30 years; well built man, a native of Guamote (highland). Had lived in Guayaquil for 8 months.

Onset Sept. 28, 1918. Sudden fever with chills, headache, pains in the limbs and back. Admitted to hospital on Sept. 30. Still complained of pains in the body and headache, with injection of the conjunctivæ; tongue coated, with free edge and tip; gums swollen. Albumin and casts in the urine, which was diminished in volume. Oct. 1. Scleras and skin somewhat yellowish. Oct. 2. All symptoms aggravated. Oct. 3. Chest showed several pea-sized hemorrhagic spots, slightly raised. Extensive ecchymotic areas were found on the skin where the patient had applied mustard.[13] Jaundice marked; gums bleeding. Frequent emission of black vomit. The blood was transmitted to guinea pigs at 2.30 p.m., Oct. 4; condition becoming worse; anuria. Oct. 5, 3 p.m. Died.

*Autopsy.*—Performed 2 hours after death. Intense jaundice throughout the entire body. Skin over the chest showed numerous dark hemorrhagic spots. Lungs much congested, showing many hemorrhagic spots. Liver bright yellowish in color, friable, and fatty. Kidneys highly congested, with cloudy swelling; some small ecchymotic foci in the pelvis. Capsules not adherent. Stomach and intestines hyperemic with blackish bloody contents. Heart showed a few ecchymoses in the pericardium and endocardium; muscles degenerated. Bladder contracted with small amount of dark yellow urine. Spleen, pancreas, suprarenal glands, and testes apparently unchanged.

*Animal Inoculation. Guinea Pig 790* (*Text-Fig. 2, d*).—Oct. 3, 1918, 2.32 p.m. Injected intraperitoneally with 3 cc. of blood from Patient Ch., on the 6th day of disease. Temperature rose to 40.2°C. on the 6th and 39.8° on the 7th day

[13] See Fig. 1, Paper I.




TEXT-FIG. 2, *a* to *d*. Direct transmissions from patient to guinea pig by means of injection of the blood of Patient Ch., Case 5. (*a*) Case 5. Age 30 years. (*b*) Guinea Pig 790 B. Strain, Case 5. (*c*) Guinea Pig 790 A. Strain, Case 5. (*d*) Guinea Pig 790. Strain, Case 5.

and came down to 37° on the 8th day, when the scleras and skin were intensely jaundiced. The animal was rapidly weakening and was killed for examination of the lesions and also for cultivation of material.

*Autopsy.*—Epistaxis; hemorrhagic lesions in the lungs and gastrointestinal tract. Liver pale yellow and fatty, and kidneys and suprarenal glands congested and swollen. Other organs apparently unchanged. In the blood and emulsions of the liver and kidneys the leptospiras were demonstrated. A pure culture was obtained from the blood of this animal.

*Guinea Pig 791.*—Duplicate of the foregoing experiment. The animal did not become infected.

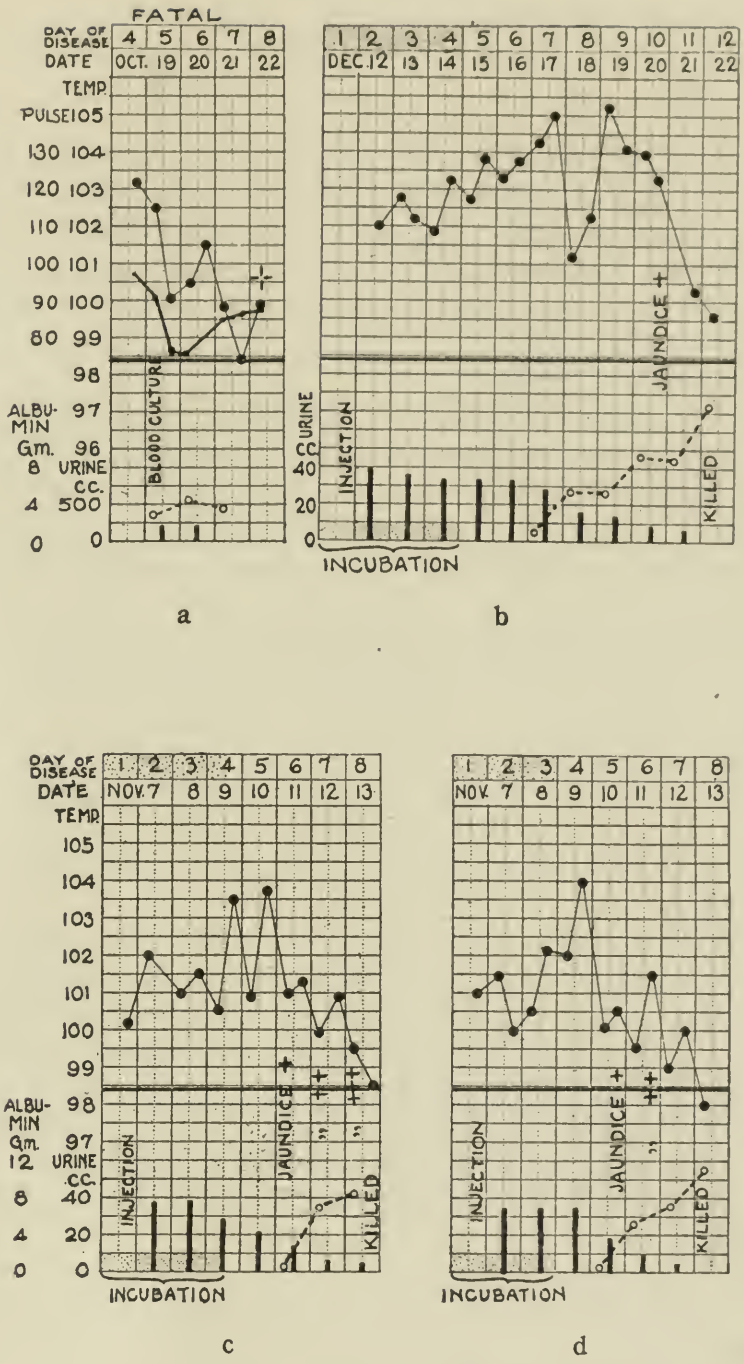
*Passage.*—Two guinea pigs were inoculated intraperitoneally on Oct. 11 with 1 cc. of blood from Guinea Pig 790, and both came down with typical symptoms and lesions within 8 days (Text-fig. 2, *b* and *c*). The leptospiras were demonstrated in the blood and emulsions of the liver and kidneys. Pure cultures were obtained from the blood of these guinea pigs.

The seventh positive transmission was obtained with a culture derived directly from the blood of Patient A. Ce.

*Case 6* (*Text-Fig. 3, a*).—A. Ce., female, age 18 years; seamstress, from a coast town of Vinces. Had been in Guayaquil 12 days.

Onset with chills and fever on Oct. 16, 1918, followed by headache, pains in the back, legs, and epigastric region. Vomited yellowish matter. Admitted on Oct. 18. Slight jaundice; marked albuminuria; intense pain throughout the body; nausea. Several petechial hemorrhages on the chest and arms. Oct. 19. Jaundice more distinct; tongue coated, with free edge and tip; bilious vomit; albuminuria; oliguria. Patient gravely ill. Blood culture made. Oct. 20. All symptoms becoming worse. Black vomit and profuse bleeding from the gums. Oct. 21. Comatose all day. Oct. 22, 8 a.m. Died. Autopsy not performed.

The culture tubes were examined on October 26, 1918, 7 days after they were set up, by the dark-field microscope. Only one of the six tubes showed the presence of active leptospiras in small numbers. From this twelve subcultures were made. The other tubes remained apparently without growth. Inoculations into guinea pigs were made at three different laboratories, first in the laboratory of the Yellow Fever Hospital in Guayaquil on October 27, then in the laboratory of the Colon Hospital, Panama Canal, on November 7, and finally in the laboratory of The Rockefeller Institute on December 11 and 19; that is, 8, 17, 44, and 52 days respectively after the time of making the cultures. Subsequent subcultures




TEXT-FIG. 3, *a* to *d*. Indirect transmission from patient to guinea pig by means of culture derived from the blood of Patient Ce., Case 6. (*a*) Case 6. Age 18 years. (*b*) Guinea pig. Strain, Case 6. Second passage from culture. (*c*) Guinea pig. Strain, Case 6. Culture 2. (*d*) Guinea pig. Strain, Case 6. Culture 3.

of this strain have since been obtained (Figs. 5 and 6). The protocols of some of these series of inoculations of guinea pigs with the cultures follow.

*Animal Inoculation* (*Second Series*). *Guinea Pig CeI*$_2$ (*Text-Fig. 3, c*).—Nov. 7, 1918. Inoculated intraperitoneally with 0.5 cc. of the culture (17 days old) of blood from Patient Ce. Temperature 39.8°C. on the 4th day, 40° on the 5th, 39° on the 6th, and 37.2° on the 7th. Suffusion of the conjunctivæ, ears, and soles. Slight jaundice appeared on the scleras, ears, and skin on the 7th day. The urine contained bile pigment and albumin. The animal was killed on Nov. 13 for examination and culture.

*Autopsy*.—Moderate general jaundice. Many spots of hemorrhage in lungs; liver congested and brownish dark red; kidneys swollen and cloudy; gastrointestinal mucosa hyperemic and somewhat hemorrhagic; spleen not altered.

*Guinea Pig CeI*$_3$ (*Text-Fig. 3, d*).—The same as the foregoing experiment. The animal showed symptoms and lesions almost identical with those just described.

*Animal Inoculation* (*Third Series*). *Guinea Pig 4, Second Passage from No. CeI*$_2$ (*Text-Fig. 3, b*).—Dec. 11, 1918. Inoculated intraperitoneally with 0.5 cc. of the liver emulsion of guinea pig infected with the first generation of culture of Ce. strain (35 days old) which had never been passed through any animal. Temperature 39.5°C. on the 4th, 39.8° on the 5th, 40.6° on the 6th, 38.8° on the 7th, 41° on the 8th, 40° on the 9th, 38° on the 10th, and 37.6° on the 11th day. The animal became intensely yellow throughout the entire body and was killed for culture and examination.

*Autopsy*.—All tissues were deeply jaundiced, and there were some minute ecchymoses in the subcutaneous and muscular tissues. The lungs showed large and small irregular hemorrhagic spots which on section were seen to extend into the parenchyma. The color of the liver was strikingly yellow. Kidneys swollen and on section cloudy; marking indistinct. Substance of kidney deeply jaundiced. Suprarenal glands congested. Mucosa of the gastrointestinal tract hyperemic. Some hemorrhages, staining the contents blackish red. Spleen not visibly affected. Only a few leptospiras found in the kidneys and none in the blood or liver.

In addition to the animals given above five more guinea pigs were inoculated in the same series. All showed symptoms and lesions similar to those just outlined. Fig. 4 shows the organism in a stained blood preparation from one of the infected guinea pigs.

The instance just described confirms the results obtained in the first positive transmission experiment (Case A.); namely, that the leptospira can be directly cultivated from the blood of yellow fever patients and then inoculated into guinea pigs with reproduction of

the symptoms and lesions which characterize the disease in man. As we shall note again elsewhere, the virulence of this organism was maintained in cultivation for more than 5 weeks.

To sum up the results recorded in the preceding series of experiments, it is shown that there exists in certain cases of yellow fever prevalent in Guayaquil a definite organism which is capable of being transmitted to the guinea pig and reproduces the symptom complex characteristic of yellow fever. As other experiments showed, the organism which has been observed and isolated in Guayaquil is a leptospira (Figs. 1 to 6) closely resembling the *Leptospira icterohæmorrhagiæ* of Inada and Ido,[10] discovered by them in the infectious jaundice of temperate climates. That the present organism is closely allied to but immunologically distinct from that species of the infectious jaundice has since been established and the experiments bearing on this point will be discussed in detail in subsequent papers. For the present I shall refer to the organism isolated from the yellow fever (typhus icteroides) cases as the yellow fever leptospira, or *Leptospira icteroides* (ἴκτερος, jaundice, + εἰδης, resembling, like), thus denoting the source from which it was obtained.

*Negative and Abortive Infections.*

In the twenty-one instances to be recorded there was either a temporary febrile reaction in the guinea pigs after inoculation of the blood, with or without any suspicion of jaundice, or almost no reaction at all. In other words, the results are classed as indefinite or negative. It is noteworthy, however, that a considerable proportion of the guinea pigs inoculated with the blood drawn during the first 5 days of the disease had a febrile reaction either on the 4th, 5th, 6th, or 7th day, and in some of these animals even a trace of jaundice was noted or suspected for a day or two soon after the fever. In none did the jaundice become definite, and the animals subsequently returned to normal. Undoubtedly, by transferring the virus to normal animals at this stage more strains might have been secured in passage, but much time was lost in watching further development of the symptoms, and opportunities to obtain the strains were lost. Some of these animals must have had a mild or abortive

TABLE I.

| Case No. | Sex. | Age. | Course of disease. | Day of disease on which blood was taken. | No. of guinea pigs inoculated. | Outcome of inoculation. |
|---|---|---|---|---|---|---|
| | | *yrs.* | | | | |
| 7 | Male. | 21 | Severe; recovery. | 2nd | 2 | Febrile reaction only. |
| 8 | " | 21 | " " | 2nd | 2 | " " in one, fever and trace of icterus in other; recovery in both. |
| 9 | " | 19 | Moderate; " | 2nd | 2 | No reaction in one, fever and trace of icterus in other; recovery in both. |
| 10 | Female. | 26 | Severe; died on 7th day. | 2nd | 2 | No reaction. |
| 11 | " | 10 | Extremely mild. | 2nd | 2 | " " in one, slight fever in other. |
| 12 | " | 40 | Moderate; recovery. | 2nd | 2 | Febrile reaction in both. |
| 13 | Male. | 35 | Mild; " | 3rd | 2 | " " " " |
| 14 | " | 23 | Severe; " | 3rd | 2 | No reaction in one, fever in other. |
| 15 | " | 25 | Very mild; " | 3rd | 2 | Fever in both. |
| 16 | " | 25 | Moderate; " | 3rd | 2 | " " " and suspicion of icterus; both recovered. |
| 17 | " | 19 | Severe; died on 6th day. | 3rd | 1 | Fever and suspected trace of icterus; recovered. |
| 18 | " | 23 | Severe; died on 7th day. | 4th | 1 | Fever and suspicious yellow tint in scleras; recovered. |
| 19 | " | 25 | Severe; recovery. | 4th | 2 | Febrile reaction in both and suspicion of icterus in one. |
| 20 | " | 20 | " " | 4th | 2 | Fever in both and perhaps a trace of icterus. |
| 21 | " | 28 | Severe; died on 4th day. | 4th | 3 | All had a febrile reaction but no icterus. |
| 22 | " | 16 | Severe; died on 10th day. | 5th | 2 | Both had fever and suspiciously yellow scleras. |
| 23 | " | 16 | Moderate; recovery. | 5th | 1 | Febrile reaction. |
| 24 | " | 20 | Severe; died in 6 (?) days. | 5th (?) | 2 | Both had fever. Suspicion of icterus in one. |
| 25 | Female. | 21 | Mild; recovery. | 6th | 2 | Fever in one, no reaction in other. |
| 26 | Male. | 21 | Severe; " | 8th | 2 | No reaction in either. |
| 27 | " | 32 | " died on 16th day. | 14th | 2 | " " " " |
| 27 | " | 32 | Severe; died on 16th day. | 15th | 2 | " " " " |

form of the infection, as they subsequently proved to be refractory to a virulent virus when tested after a period of about 25 days from the time of the inoculation of the yellow fever blood. For the sake of completeness the protocols of the negative transmission experiments are recorded in Table I.

## SUMMARY.

By injecting into guinea pigs the blood of yellow fever cases occurring in Guayaquil a group of symptoms and lesions closely resembling those observed in human yellow fever were induced in a limited number of instances. Of 74 guinea pigs inoculated with specimens of blood from 27 cases of yellow fever, 8, representing 6 cases, came down with the symptoms; namely, a marked rise of temperature after a period of incubation averaging 3 to 6 days, with simultaneous suffusion of the capillaries, particularly of the conjunctivæ and soles, then preliminary hyperleucocytosis followed by progressive leucopenia, the early appearance of albumin and casts in the urine, which gradually diminishes in volume as the disease progresses. The fever lasts only a few days, rapidly dropping first to the normal and then usually to subnormal. At this period jaundice manifests itself in varying degrees of intensity, first in the scleras, then in the skin and the urine. Hemorrhages from the nasal or gingival mucosa or anus have been observed to occur during this period. Autopsies reveal deep jaundice throughout the entire tissue. The liver is fatty and yellow, the kidney hyperemic, and often swollen and hemorrhagic. Hemorrhagic spots were almost always found in the lungs and gastrointestinal mucosa. Guinea pigs are usually rather sensitive to the infection, though many appeared to be somewhat resistant and some even refractory.

The injection of the yellow fever blood into ringtail monkeys, rabbits, cats, guatusas, weasels, and sloths among the mammalians, and pigeons, ground-doves, bluebirds, mantas, blackbirds, parrakeets, reedbirds, blancos, and toucans among the birds, gave negative results.

In the blood, liver, and kidneys of the guinea pigs experimentally infected with the blood of yellow fever patients a minute organism

was demonstrated which closely resembles in morphology the causative agent of infectious jaundice (*Leptospira icterohæmorrhagiæ*).

The leptospira transmitted from yellow fever cases to guinea pigs was found to induce similar symptoms and lesions upon further passage into normal guinea pigs.

The leptospira obtained from cases of yellow fever has been given the provisional name of *Leptospira icteroides*.

I wish to express my thanks to Dr. León Becerra, General Director of the Department of Health of Ecuador, and his staff for their support and cooperation in the execution of this work; and likewise to Dr. Pareja of the Guayaquil Yellow Fever Hospital and to his laboratory staff (Dr. Larrea), clinical staff (Dr. Davila and Dr. Martinez), and nursing staff for their invaluable assistance.

To Dr. Herman B. Parker I am indebted for his courtesy in lending me a dark-field arc lamp during the period before my own arrived, when the dark-field work would otherwise have been impossible, and also in furnishing me on various occasions with clinical material.

I am indebted to Colonel McCormack, Chief Health Officer of the Canal Zone, to Major Teague and Captain McFarland of the Ancon Hospital, and to Captain Bowen and Lieutenant Levy of the Colon Hospital, for the facilities afforded me in making renewal of cultures and transfers of strains to new animals.

## EXPLANATION OF PLATE 35.

FIG. 1. *Leptospira icteroides* in the blood of a guinea pig experimentally inoculated with culture of strain from Patient A. A., Case 1. Fixed in methyl alcohol and stained with Wright's stain. The film was made on the 6th day of illness. × 1,000.

FIG. 2. The same; strain from Patient M. G., Case 3. × 1,000.

FIG. 3. The same; strain from Patient Co., Case 4. × 1,000.

FIG. 4. The same; strain from Patient A. Ce., Case 6. × 1,000.

FIG. 5. Dark-field view of a culture 16 days old of *Leptospira icteroides*. Strain from Patient A. Ce., Case 6. × 1,000.

FIG. 6. The same. × 1,000.

# ETIOLOGY OF YELLOW FEVER.

## III. Symptomatology and Pathological Findings in Animals Experimentally Infected.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 36 to 38.

(Received for publication, March 26, 1919.)

Mention has already been made[1] of successfully inducing in guinea pigs symptoms and pathological conditions simulating those observed in yellow fever patients in Guayaquil by inoculating these animals with the blood or organ emulsions from yellow fever patients. It has also been stated[1] that in the blood and organ emulsions of the infected guinea pigs an organism belonging to the genus *Leptospira* has been demonstrated and that the organism, after having been obtained in culture, is capable of inducing the same symptoms and pathological changes in these animals as does the original blood of yellow fever patients. In this paper the mode and course of this infection as observed in guinea pigs, dogs, and monkeys will be described.

### *Mode of Experimental Infection.*

Infection with this organism may be induced either by injection into the peritoneal cavity, the blood circulation, or the subcutaneous tissues, or by application to the scarified, depilated surface of the skin or to the mucous membranes, or by feeding the animal with infected tissue or culture.

### *Experimental Infection in Guinea Pigs (Text-Fig. 1, a to f).*

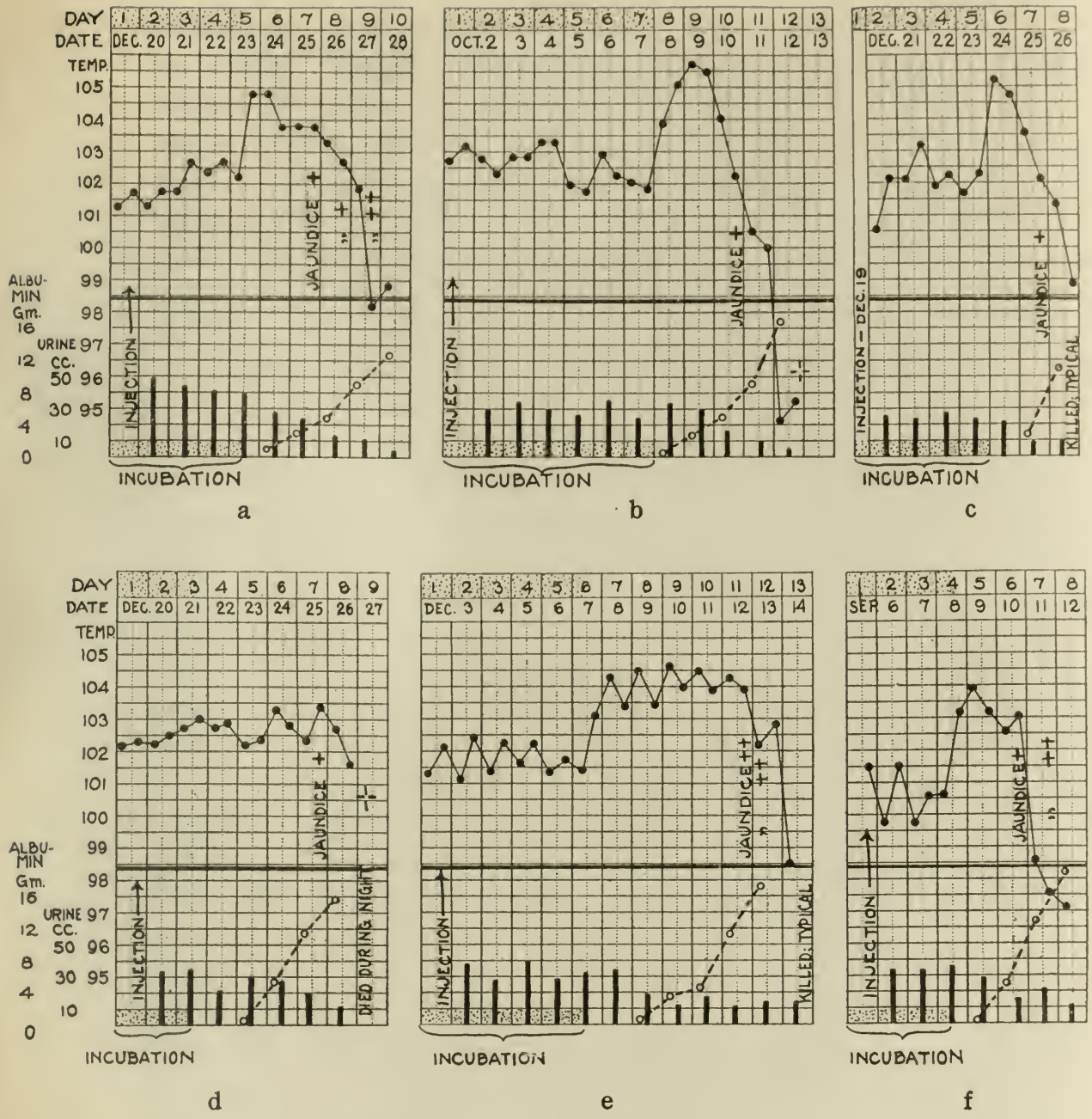
*Incubation Period.*—The incubation period varies according to the mode of infection and the quantity of virus introduced. When a large

[1] Noguchi, H., Etiology of yellow fever. II. Transmission experiments on yellow fever, *J. Exp. Med.*, 1919, xxix, 565.

amount is inoculated intraperitoneally or into the circulation the first symptoms—inactivity, anorexia, hyperleucocytosis—make their appearance after 48 hours, followed by a rise in temperature and slight albuminuria within the next 24 hours. With subcutaneous inoculation the symptoms do not appear until the 4th (72 hours) or 5th (96 hours) day, and with a very small amount of the virus a few days later. The percutaneous and *per os* modes of infection require a period of about 6 and sometimes as many as 12 days of incubation.

*Onset and Course.*—The onset is indicated by loss of appetite, inactivity, injection of the bulbar conjunctivæ, and a sudden rise in temperature ranging from 39.8–40.5°C. and rarely 41°C. The animal offers little resistance to handling, plaintive cries indicating intense muscular pains. The urine diminishes in quantity from an average daily output of about 25 cc. to almost half that, and its color changes from a pale straw tinge to dark brownish yellow. It now contains a moderate amount of albumin, with some epithelial cells and granular casts. At this stage no bile pigment can be demonstrated in the blood serum and no jaundice in the scleras or other parts of the body. The leucocytes are increased during the 1st day, but leucopenia follows within a few days, the differential count showing a marked increase of polymorphonuclear leucocytes.[2] Within the next 24 hours the temperature shows a slight drop, only to return to 39.8–40°C. during the following day. The fever then subsides gradually until the temperature drops below the normal in 3 or 4 days more. In fatal cases it sometimes drops to 34°C. just before death. Although the temperature begins to fall on the 2nd day all the other symptoms are aggravated. The urine is reduced still further in quantity and contains an enormous amount of albumin, renal epithelia, granular and hyaline casts, and the bile pigment first appears. At the same time the scleras become deeper yellow and are intensely suffused. At this stage there is a trace of bile pigment in the serum. On the 3rd day icterus becomes quite distinct

[2] I am greatly indebted to Dr. Jorge Larrea, Director of the Laboratories of the Guayaquil Yellow Fever Hospital, for total leucocyte counts on some of the experimental animals. My thanks are also due to Dr. F. Rojas of the General Hospital in Guayaquil.




TEXT-FIG. 1, *a* to *f*. Observations on guinea pigs inoculated with the cultures of the different strains of *Leptospira icteroides*. (*a*) Guinea pig. Strain, Case 5. (*b*) Guinea pig. Strain, Case 5. (*c*) Guinea pig. Strain, Case 6. (*d*) Guinea pig. Strain, Case 5. (*e*) Guinea pig. Strain, Case 6. (*f*) Guinea pig. Strain, Case 1.

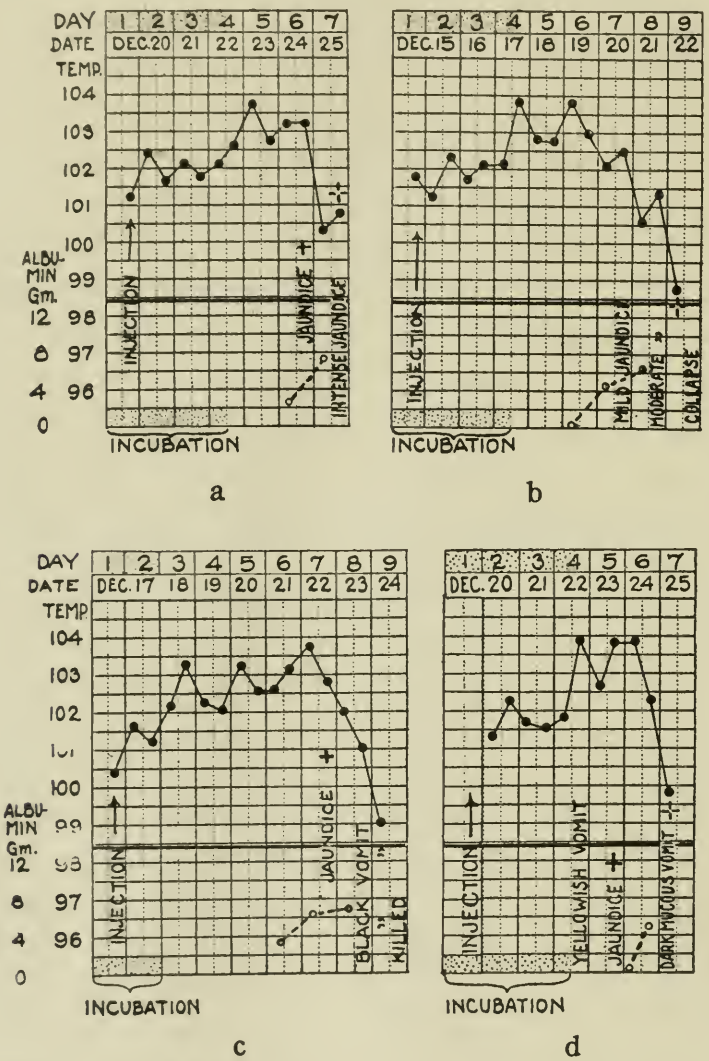




in the ears, scleras, palms, and soles. There may be ecchymoses in the scleras. Epistaxis and hematuria are frequent symptoms. The urine is now a deep grayish brown hue, and only a few cubic centimeters may be excreted. The animal still keeps on its feet but is quiet. On the 4th day the jaundice becomes general, and the serum taken is deep brownish yellow, as is also the urine. Erythrocytes, renal cells, albumin, and bile pigment are very abundant in the scanty urine which may still be passed, or there may be total anuria. Melena or bleeding from the rectum has been observed, and epistaxis is a frequent phenomenon in a dying animal (Fig. 13). Death occurs between the 5th and 7th days after onset of the disease and is accompanied either by clonic convulsions or by gradual asphyxiation, with air-hunger. The blood pressure becomes so low that about 2 days before death very little or almost no blood flows from the largest ear veins, even when they are completely cut across.

*Non-Fatal and Abortive Infections.*—Experimental yellow fever in the guinea pig is not always fatal. A certain proportion of the animals inoculated with a virulent strain of the virus show a temporary febrile reaction and albuminuria with few casts. Jaundice is slightly noticeable in the ears (if the animal is white or light in color) and scleras, but it is sometimes apparently absent. The fever and other symptoms set in as in fatal cases but completely pass away within a few days. In a week the animal has regained its previous health. That these reactions are mild or atypical symptoms of the same infection is evident from the fact that such animals cannot be reinfected, even with a quantity of the virus sufficient to kill control guinea pigs with the typical symptoms and lesions. Furthermore, these mild infections occur among guinea pigs inoculated with the same quantity of virus which produces a fatal infection among others in the same group, and especially do they occur frequently when the amount of the virus injected is rather small or the strain attenuated in virulence for the guinea pig. As in yellow fever in man, therefore, so in the experimental condition in guinea pigs, there apparently exist varying grades of severity of infection according to the individual resistance to the same virus. Many instances have been encountered, among many hundreds of guinea pigs purchased in Guayaquil, in which the animals showed a complete immunity to the

inoculation of the virus. It is possible that as some of them had been kept in houses or markets for varying periods of time before purchase they were rendered immune through a previous mild infection.




TEXT-FIG. 2, *a* to *d*. Observations on dogs inoculated with the cultures of the different strains of *Leptospira icteroides*. (*a*) Dog (pup). Strain, Case 6. (*b*) Dog (pup). Strain, Case 1. (*c*) Dog (pup). Strain, Case 5. (*d*) Dog (pup). Strain, Case 3.

*Experimental Infection in Dogs (Text-Fig. 2, a to d).*

*Incubation Period.*—This is practically the same as in guinea pigs.

*Onset and Course.*—The onset and course are also similar to those observed in guinea pigs, some dogs succumbing to the infection and

others recovering from a mild attack. In a fatal infection the temperature may reach 40°C., but usually it is not over 39.5°C., and lasts for 3 or 4 days, when it gradually drops by lysis. The skin then becomes yellowish and the conjunctivæ injected, and the animal refuses to eat. It often vomits bilious and sometimes blackish, mucous, frothy contents from the stomach. The amount of urine diminishes, with increasing albumin, casts, and bile pigments. Death occurs in coma or in clonic convulsions, the temperature falling below normal.

*Experimental Infection in Monkeys (Text-Fig. 3, a to e).*

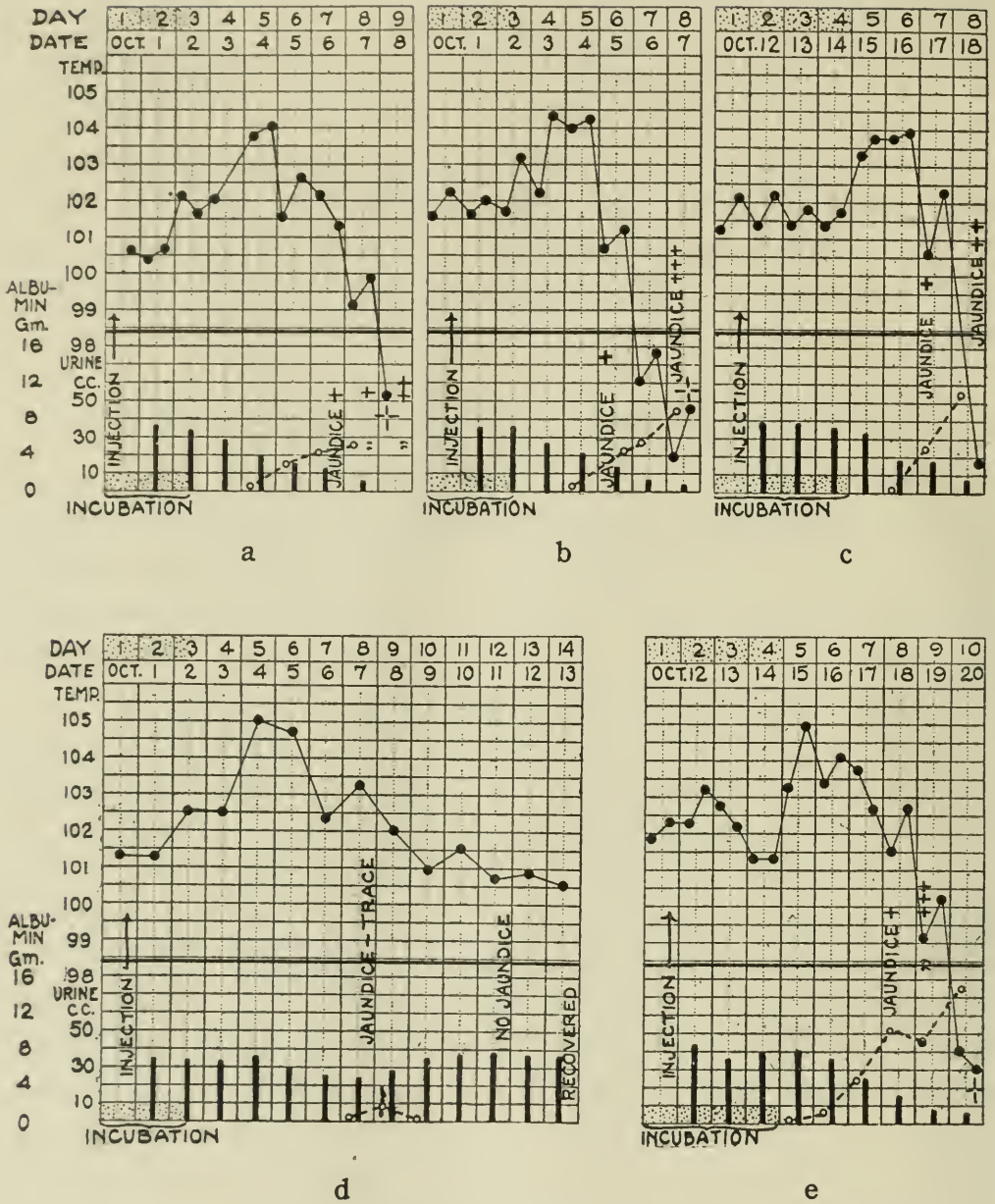
As stated elsewhere, the South American ringtail monkeys were found refractory to infection with the blood of yellow fever patients or the culture of the yellow fever leptospira.[1] With both a temporary febrile reaction was observed, but the animal invariably recovered. Five marmosets,[3] however, proved to be susceptible to the organism, four being fatally and one mildly infected.

The incubation period varied from 3 to 4 days, during which time the animals appeared well. The disease became noticeable by their inactivity, loss of appetite, non-resistance to handling, and slight greenish pigmentation of the urine. Albumin, casts, and bile pigments began to appear in the urine when the fever reached its height. Mild jaundice in the scleras, abdominal skin, and oral mucous membrane became noticeable a day or two after the fever began to subside. On the day of death the animals lay in a helpless position, offering only feeble resistance to handling. Death occurred during a condition of general weakness, coma, and sometimes convulsions. The temperature was usually subnormal, and the body of the animal more distinctly and generally yellowish.

*Autopsy Findings in the Experimental Infection (Fig. 14).*

The guinea pigs, dogs, and marmosets dying of the experimental infection invariably show a pronounced jaundice ranging from a light yellow to a deep bright yellow. Blood clots or stains are often

[3] Species *Midas œdipus* and *Midas geoffroyi.*




TEXT-FIG. 3, *a* to *e*. Observations on monkeys inoculated with the different strains of *Leptospira icteroides*. (*a*) Monkey 1. Strain, Case 1. Culture of second generation, 2 cc. intraperitoneally. (*b*) Monkey 2. Strain, Case 3. Culture of first generation, 4 cc. intraperitoneally. (*c*) Monkey 5. Strain, Case 6. Guinea pig kidney emulsion, 6 cc. intraperitoneally. (*d*) Monkey 3. Strain, Case 1. Culture of second generation, 4 cc. intraperitoneally. (*e*) Monkey 4. Strain, Case 5. Guinea pig liver emulsion, 6 cc. intraperitoneally.

present in the nostrils, mouth, anus, or vagina. Subcutaneous ecchymoses are frequently present in the guinea pig but seldom in the dog or marmoset. Postmortem rigor and lividity are marked. In guinea pigs the subcutaneous tissues are intensely jaundiced, and ecchymoses are seen in the axillary and inguinal regions. The abdominal muscles are often spotted with minute ecchymoses but sometimes these are absent. In the dog and marmoset the muscles show very slight or no hemorrhage.

The lungs of the guinea pig almost always show ecchymoses varying in extent from few and minute to numerous and large ones, roughly round, oval, or irregularly oblong or square, and with or without a sharp outline, vivid red, dark, or bluish red in color, sometimes light red with a darker center. Undoubtedly the color of the spots becomes darker and more bluish as they become older, since the longer the animal lives the darker are these ecchymoses. Postmortem hypostasis is marked. There may be some ecchymoses in the pleuræ. In the dog and marmoset the lungs are much less affected, only a few spots of hemorrhage being found, and the pleuræ are usually free.

There may be some dilation of the right heart. The pericardium is often studded with minute hemorrhagic spots. The fluid is clear and icteric. The muscle is friable and brownish yellow, and there are few or sometimes many ecchymoses on the surface. The endocardium and papillary muscles seem normal except for occasional punctiform ecchymoses. The valves are not altered but are yellowish in color, the aorta usually being deeply jaundiced. These changes apply equally to the guinea pig, dog, and marmoset.

The liver is usually slightly enlarged and varies in color from a yellowish brown-red to a bright yellowish brown. In instances in which death occurred within the first 3 days the degeneration was less advanced and the yellowish color not so pronounced. The surface is often mottled or striped with yellowish brown and brownish red, and the markings are very distinct.

The gall bladder is usually filled with a dark green or greenish yellow bile, and the wall is often spotted with minute ecchymoses.

The stomach usually contains some undigested food which is mixed with blood from the adjacent ecchymotic area of the mucosa. The contents are sometimes semifluid containing blackish blood re-

sembling coffee-grounds in appearance. In dogs and marmosets the greater quantity of mucus renders the appearance of the stomach contents indistinguishable from those found in human autopsies. The mucosa is somewhat hyperemic, and numerous ecchymoses are found, especially near the cardia. The serosa of dogs and marmosets is free of hemorrhages.

The small intestine and colon, including the rectum, are intensely injected, and numerous hemorrhages are found in the mucosa. The serosa is sometimes affected. The contents are blackish in color and may contain freshly escaped blood (melena). In the dog the character of the intestinal contents is a closer reproduction of what is observed in man, the serosa in this animal not showing the ecchymoses which are present in the guinea pig or any appreciable fluid in the peritoneal cavity. The nature of the findings in marmosets is about midway between that of guinea pigs and that of dogs.

In guinea pigs the kidneys are extensively involved. Hyperemia and punctiform hemorrhages in the parenchyma are almost constant. The ecchymoses, however, vary from a few to almost countless numbers in extreme instances. In dogs and marmosets there may be only a few minute spots. On section the cortex is broader than normally and highly hyperemic, with cloudy swelling. The medullary portion is succulent and hyperemic near the border. Bloody fluid or clot may be found in the pelvis, and sometimes numerous punctiform ecchymoses.

In the guinea pig hyperemia and hemorrhages were found in the suprarenal glands, but in dogs and marmosets only a comparatively slight degree of hyperemia. There were no changes in the pancreas or spleen, except a slight enlargement of the latter in rare instances.

The lymphatic glands show, in guinea pigs, general adenopathy, with occasional hemorrhages. In dogs and marmosets some glands only are enlarged and congested.

The bladder frequently contains bile-stained urine full of albumin, casts, and cells.

The testicles are apparently not affected, although in the guinea pig hemorrhages are frequent in the adipose tissue around them.

The ovaries are usually congested, and the uterus very much so, and in the pregnant state there were hemorrhages into the amnionic

fluid. The endometrium is congested, sometimes with a clot in the cavity.

No gross changes are observed in the nervous system. The membranes are hyperemic and the fluid is icteric.

*Histological Examination (Figs. 1 to 12).*

*Lungs (Figs. 3 and 6).*—The areas of hemorrhage are most abundant and extensive in the guinea pig. The alveoli in the hemorrhagic areas are completely filled with blood corpuscles, and in the adjacent zone a marked degree of edema is evident. There are also small accumulations of polymorphonuclear leucocytes and endothelial cells. In marmosets and dogs these changes are far less extensive. The leptospiras were demonstrated in the tissues by the Levaditi method.

*Liver (Figs. 1 and 4).*—The degree of degeneration of the liver cells is variable in different animals. In well marked instances the majority of the cells are swollen and necrotic. Vacuoles are found in some cells. The nuclei are swollen and degenerated. In some areas the liver cells are dissociated, swollen, and appear to have lost their sharp hexagonal outlines. The liver cells near the blood vessels seem to be less affected than those around the portal canal. Hemorrhagic foci of varying dimension are distributed irregularly. The endothelial cells of the bile ducts are increased in size, and there are some lymphoid cells around the portal canal zone. Mitotic figures are found. The organisms were found in the tissues stained by Noguchi's method (Figs. 7 and 10).

*Kidneys (Figs. 2 and 5).*—The epithelium of the convoluted tubules shows granular, swollen, and sometimes vacuolated cytoplasm. The cells may be detached from the membrane and fill up the lumen, which is distended with granular and hyaline casts. The glomeruli are considerably injected, and numerous hemorrhages are found throughout the cortex and medulla (Figs. 8, 9, 11 and 12).

*Stomach.*—There are superficial congestion of the mucosa and some hemorrhagic foci. In certain areas there is an accumulation of lymphoid and plasma cells.

*Large and Small Intestines.*—The intestines show injection and occasional hemorrhages.

*Heart.*—Certain fibers are swollen and contain vesicular nuclei.

*Spleen.*—There are hemorrhages, and the pulp is rich in blood.

*Lymph Nodes.*—These show phagocytosis and central degeneration of the follicles.

*Adrenal.*—In guinea pigs there are parenchymatous degeneration, congestion, and hemorrhages in some instances. These changes are less frequent in dogs and marmosets.

*Pancreas.*—Little change.

*Nervous System.*—Little change.

## SUMMARY.

Studies are reported on the type of disease induced in guinea pigs, dogs, and monkeys by inoculating them (1) with the blood or organ emulsions of guinea pigs or other susceptible animals experimentally infected with *Leptospira icteroides*, and (2) with a pure culture of the organism. Particular attention has been given in these experiments to the clinical features of the experimental infection in the various animals and to the pathological changes resulting from the infection.

The symptoms and pathological lesions induced in guinea pigs are much more pronounced than those observed in dogs or marmosets. The period of incubation is nearly the same in all three species, 72 to 96 hours with intraperitoneal or subcutaneous inoculation, and a day or more longer when the infection is induced percutaneously or *per os*. The febrile reaction in the guinea pig and marmoset is about the same; in the dog there is less fever. The amount of albumin, casts, and bile pigments in the urine is more abundant in the guinea pig and marmoset than in the dog, and these animals also appear on the whole to become more intensely icteric. The black or bilious vomit, however, though occurring frequently in dogs during life, is observed in the guinea pig and marmoset at autopsy. The hemorrhagic diathesis is most pronounced in guinea pigs, less so in marmosets, and least in dogs. In dogs, for example, subcutaneous hemorrhages almost never occur, and the lungs usually show only a few minute ecchymoses. The pleuræ, pericardium, and other serous surfaces of the thorax and abdomen remain free from ecchymoses,

which, however, with hyperemia, are very marked along the gastro-intestinal tract.

The symptoms and lesions observed in animals experimentally infected with *Leptospira icteroides* closely parallel those of human yellow fever.

The pathological changes occurring in human cases of yellow fever are similar to those induced by inoculation in guinea pigs and marmosets and in respect to their intensity stand intermediate between those arising in the two animals mentioned.

## EXPLANATION OF PLATES.

### PLATE 36.

Sections of tissues from yellow fever cases, fixed in Zenker's fluid and stained with eosin and methylene blue. × 150.

FIG. 1. Section of the liver of a marmoset inoculated with the organism isolated from Patient E. Ch., Case 5, showing hemorrhage and necrosis, vacuolization, and dissociation of the liver cells.

FIG. 2. Section of the kidney from the same marmoset, showing necrosis, detachment, and some vacuolization of the renal epithelia. Some of the lumina are seen to contain granular casts. The glomeruli seem to be highly congested; hemorrhage in some portions of the tissue.

FIG. 3. Section of the lung from the same marmoset, showing hemorrhage in the tissue.

FIG. 4. Section of the liver from a guinea pig inoculated with the organism isolated from Patient M. G., Case 3. The liver cells are seen to have been largely disintegrated and replaced by red corpuscles and debris.

FIG. 5. Section from the kidney of same guinea pig, showing advanced degeneration of renal epithelia, with granular and hyaline casts in the lumen.

FIG. 6. Section of the lung from a guinea pig inoculated with the strain isolated from Patient A. Ce., Case 7. There is a considerable degree of hemorrhage and edema. Some leucocytic infiltration seems to be evident.

### PLATE 37.

Sections from the liver and kidney of marmosets and guinea pigs inoculated with the strains of *Leptospira icteroides* derived from Cases 1, 3, and 5. Figs. 10 to 12 are stained by the method of Levaditi, Figs. 7 to 9 by the writer's modification of that method. × 1,000.

FIG. 7. Liver of marmoset, showing the organism between the hepatic cells; strain from Case 5.

FIG. 8. The same strain. There are two minute, delicate organisms in the lumen of a kidney tubule.

FIG. 9. The same.

FIG. 10. The organism in the guinea pig liver, in which it appears somewhat coarse.

FIGS. 11 and 12. The organism in the kidney; coarse and quite numerous.

## PLATE 38.

FIG. 13. Epistaxis in a guinea pig inoculated with a culture of *Leptospira icteroides* isolated from Patient A. Ce., Case 6. The picture was taken at the moment of death.

FIG. 14. Autopsy of a guinea pig killed on the 6th day after inoculation with a culture of *Leptospira icteroides* isolated from Patient A. Ce., Case 6. The animal showed typical symptoms and was killed at the time of collapse. The picture shows the general jaundice of the skin and subcutaneous tissues and the yellowish liver. Hemorrhages are evident in the lungs, and can be seen to have taken place in the stomach, as shown by its dark color. The kidney is congested, and sometimes a few ecchymotic spots can be seen. The spleen appears to be normal. The intestines and also the muscles along the thorax, abdomen, and postperitoneal region are comparatively free from hemorrhage, presenting a striking contrast to the highly congested condition of these muscles in guinea pigs inoculated with the organism of infectious jaundice. The strikingly yellow color of the liver is far more intense than is usually observed in experimental infectious jaundice in the guinea pig.

# THE PASSAGE OF MENINGOCOCCIC AGGLUTININS FROM THE BLOOD TO THE SPINAL FLUID OF THE MONKEY.

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The administration of antimeningococcic serum for the treatment of epidemic meningitis by other than the intraspinal route has hardly been considered until recently, since the publication of the papers by Flexner and his coworkers[1,2] on the specific serum therapy of the disease on which the prevailing mode of treatment is based. The experience with a large number of cases of epidemic meningitis during the great war and under the unusual conditions of camp life has led to the revival of the employment of the serum by intravenous, usually associated with intraspinal, injection. That the intravenous administration was both indicated and called for is evidenced by the occurrence of cases of meningococcemia in some instances without, and in others before the onset of, the meningitis.[3,4] Although other clinicians have from time to time employed antimeningococcic serum by intravenous injection the systematic use of it in that manner has been developed especially by Herrick.

The purpose of the intravenous injection may be regarded as threefold: (1) to combat the meningococcemia; (2) to diminish possibly the rapidity of the passage of the serum from the subarachnoid space into the blood; and (3) to bring remote portions of the meninges, not so readily accessible from the spinal fluid itself, under the influ-

[1] Flexner, S., *J. Am. Med. Assn.*, 1906, xlvii, 560; *J. Exp. Med.*, 1917, ix, 168; 1913, xvii, 553.

[2] Flexner, S., and Jobling, J. W., *J. Exp. Med.*, 1908, x, 141, 690.

[3] Herrick, W. W., *J. Am. Med. Assn.*, 1918, lxxi, 62; *Arch. Int. Med.*, 1918, xxi, 541.

[4] Baeslack, F. W., Bunce, A. H., Brunelle, G. C., Fleming, J. S., Klugh, G. F., McLean, E. H., and Salomon, A. V., *J. Am. Med. Assn.*, 1918, lxx, 684.

ence of the serum. Under normal conditions antibodies do not pass from the blood into the cerebrospinal fluid,[5] but under circumstances of inflammation of the meninges the permeability is increased, and passage may in some degree take place. Of the three possibilities, the first, namely the control of the blood invasion by meningococci, may be regarded as the most important, since it may, if only in rare instances, prevent or abort a meningeal infection, and it may be even more effective in preventing the infections of joints, heart, eye, etc.

The experiments to be described relate to the question of the possibility and the degree of passage of antibodies, in this instance agglutinins, for the meningococcus from the blood into the cerebrospinal fluid.

## EXPERIMENTAL.

*Macacus rhesus* monkeys were employed for the experiments because of the ease with which chemical meningitis may be induced in them and especially because they readily yield cerebrospinal fluid on lumbar puncture.

The first experiment was arranged to test two points: (*a*) whether the antimeningococcus agglutinins passed from the blood into the cerebrospinal fluid in normal animals, and (*b*) whether they passed in animals in which a chemical meningitis had been incited when only 10 cc. of the antiserum had been injected into a superficial vein. The protocol which follows shows not only that no such passage takes place in the normal animal but either none that is demonstrable, or at least very little, even in the presence of an aseptic meningitis.

*Experiment 1.*—Mar. 13, 1918. Three *Macacus rhesus* monkeys, weighing about 3 kilos each, received intravenously 10 cc. of polyvalent antimeningococcic serum. Monkeys A and B had received 18 hours previously 2 cc. of normal horse serum intraspinally. No intraspinal injection was given Monkey C. 7 and 24 hours after the intravenous injection spinal fluid was removed from each and tested for agglutination against the meningococcus. None was observed in either specimen from the monkey which received no intraspinal injection. The same was true of the specimens from one of the monkeys in which chemical meningitis had been induced. The spinal fluid removed 7 hours but not 24 hours after intravenous injection from the second monkey, which had received intraspinal injection of horse serum, agglutinated regular and parameningococcus in a dilution of 1:2 and 1:4.

[5] Flexner, S., *J. Am. Med. Assn.*, 1913, lxi, 447, 1872.

The second experiment was made with 20 cc. of polyvalent antimeningococcic serum of high titer which was injected in each instance into a superficial vein. It was devised to cover the following conditions: (*a*) effect on normal cerebrospinal fluid, (*b*) effect on cerebrospinal fluid modified by a mild aseptic meningitis induced by an intraspinal injection of sterile normal salt solution, (*c*) effect on the cerebrospinal fluid modified by a more severe chemical inflammation induced by normal horse serum, and (*d*) a still more severe inflammation brought about by the injection of a salt solution suspension of regular meningococcus culture. In the last instance the agglutinin tested for was that of the para organism.

The result is clear: no agglutinins were present in the cerebrospinal fluid derived from the normal animal which received the intravenous injection; fluctuating amounts were present in the cerebrospinal fluid after salt solution, more after the horse serum injection, and most after the injection of meningococcus. In other words, the normal meninges were not permeable, while the inflamed membranes were permeable in proportion to the intensity of the meningitis experimentally induced.

*Experiment 2.—Macacus rhesus* D, weight 3.2 kilos; control. Mar. 21, 1918. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. 5.25 p.m. Lumbar puncture, 0.5 cc. of clear fluid which did not agglutinate regular or parameningococcus. Mar. 22, 10.10 a.m. Lumbar puncture, 1 cc. of clear fluid which did not agglutinate the meningococci. 5.50 p.m. Injected intraspinally one-quarter of an 8 hour culture of regular meningococcus in 0.5 cc. of isotonic salt solution. Mar. 23, 12 m. Lumbar puncture, 0.5 cc. of turbid fluid which, after centrifugation, agglutinated parameningococcus ++ in a dilution of 1:4. Culture from spinal fluid negative. Mar. 24, 10.35 a.m. Lumbar puncture, 0.5 cc. of turbid fluid. Cultures negative. Centrifuged fluid agglutinated parameningococcus + in a dilution of 1:2.

*Macacus rhesus* E, weight 3.5 kilos. Mar. 20, 1918, 4 p.m. Injected intraspinally 2 cc. of isotonic salt solution. Mar. 21, 11.10 a.m. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. Mar. 22, 10.30 a.m. Lumbar puncture, 1 cc. of slightly turbid fluid. The centrifuged fluid did not agglutinate regular or parameningococcus in a dilution of 1:2. 5.45 p.m. Injected intraspinally one-quarter of an 8 hour culture of regular meningococcus in 0.5 cc. of isotonic salt solution. Mar. 23, 11 a.m. Lumbar puncture, 0.5 cc. of turbid fluid which yielded negative culture. The centrifuged fluid agglutinated parameningococcus + in a dilution of 1:4. Mar. 24, 10.25 a.m. Lumbar punc-

ture, 0.5 cc. of slightly turbid fluid which yielded negative culture. The centrifuged fluid agglutinated parameningococcus ++ in a dilution of 1:4.

*Macacus rhesus* F, weight 3.3 kilos. Mar. 20, 1918, 4 p.m. Injected intraspinally 2 cc. of normal horse serum. Mar. 21, 10.38 a.m. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. Mar. 22, 10.20 a.m. Lumbar puncture, 0.5 cc. of turbid spinal fluid. The centrifuged fluid agglutinated regular meningococcus ± 1:4, − 1:2, and parameningococcus + 1:4 and − 1:2. 6.15 p.m. Lumbar puncture, 0.5 cc. of turbid fluid which after centrifuging agglutinated parameningococcus + 1:4. Injected intraspinally one-fourth of an 8 hour culture of regular meningococcus in 0.5 cc. of isotonic salt solution. Mar. 23, 11.30 a.m. Lumbar puncture, 0.5 cc. of turbid fluid; culture negative; the centrifuged fluid agglutinated regular meningococcus + in a dilution of 1:2, ++ 1:4, and parameningococcus + 1:2, − 1:4.

The next experiment brings out conclusively the effect of degree of chemical inflammation in promoting the passage of agglutinating bodies from the blood into the spinal fluid. The order of the experiment was first to inject intraspinally into *Macacus rhesus* monkeys either isotonic salt solution or normal horse serum, and about 20 hours later to give 20 cc. of polyvalent antimeningococcic serum intravenously. The spinal fluid is then withdrawn at stated intervals and tested. When salt solution or horse serum is injected intraspinally and the turbid fluid withdrawn later and tested for agglutinins of the meningococcus no agglutination takes place unless antimeningococcic serum has been given also. The results of this experiment are given in Table I. The far greater effect of the horse serum over isotonic salt solution is at once apparent.

Attention has been drawn to the practice now becoming more common of combining the intravenous with the intraspinal injection of the antimeningococcic serum. Time and experience alone will decide in how far this practice is superior to the intraspinal injection alone. The serum introduced into the subarachnoid space soon begins to escape into the blood; hence the necessity for its reintroduction if the meninges are to be kept bathed in the antiserum. The passage of the antiserum from the subarachnoid space into the blood leads to a general distribution throughout the organs. Possibly the higher dilution thus produced compared with the greater concentration secured from a direct injection of the larger volume of serum into the blood gives to the latter a special advantage in overcoming the so called meningococcemia.

It seemed desirable to determine, by direct agglutination tests, the agglutination titer of the blood and spinal fluid under the three sets of conditions; namely, after an intraspinal injection alone, after an intravenous injection alone, and after combined intraspinal and intravenous injection. This determination was made in monkeys; it could of course be carried out better with cases of epidemic meningitis under serum treatment. Unfortunately we were restricted by the scarcity of monkeys to one test of each condition. The result given in Text-fig. 1, *a*, *b*, and *c* must be regarded, therefore, as tentative only. Certain factors affecting the results are entirely ob-

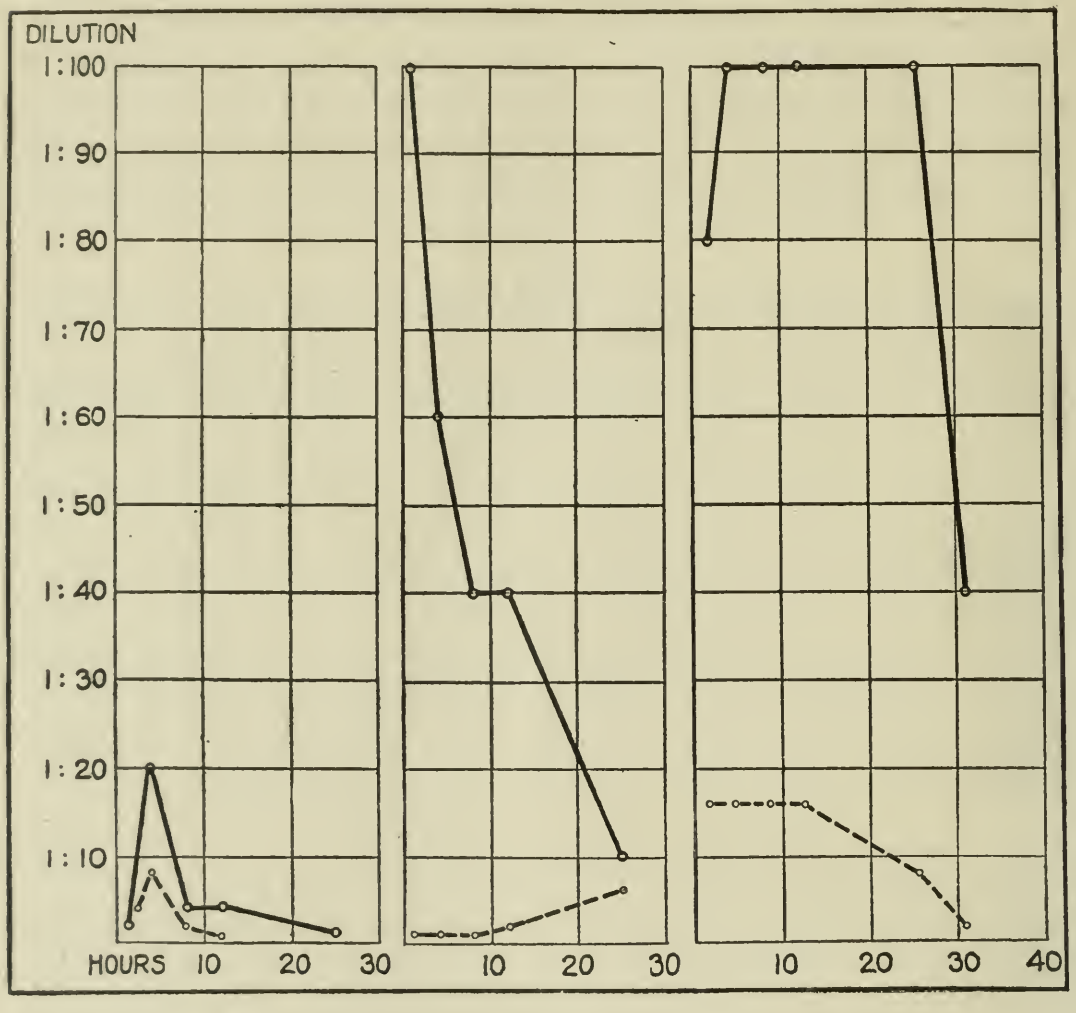
TABLE I.

| Intraspinal injection. | Length of time after intravenous injection. | Dilutions of spinal fluid. | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1:2 | 1:4 | 1:8 | 1:10 | 1:20 | 1:40 |
| | *hrs.* | | | | | | |
| | 20 | + | + | ± | − | − | − |
| | 25 | + | + | ± | − | − | − |
| Isotonic salt solution. | 45 | ± | ± | − | − | − | − |
| | 69 | ± | − | − | − | − | − |
| | 93 | ± | − | − | − | − | − |
| | 20 | + | + | + | ± | − | − |
| | 25 | + | + | + | + | ± | − |
| Normal horse serum. | 45 | ++ | ++ | ++ | + | + | + |
| | 69 | ± | + | ± | − | − | − |
| | 93 | + | + | ± | − | − | − |

scure; such, for example, as the delay after intravenous injection before the full expression of the agglutination titer declares itself. The three curves are, however, not only distinct, but suggest first that passage of the serum from the cerebrospinal fluid into the blood begins almost at once (Text-fig. 1, *a*), and second that the persistence of the titer in the blood and spinal fluid (Text-fig. 1, *c*) is sensibly affected by combined intraspinal and intravenous injection. However, we desire to repeat that not too much stress should be laid upon the results of these single tests.

*Experiment 3.—Macacus rhesus* G. May 14, 1918, 9.15 a.m. Injected intraspinally 3 cc. of polyvalent antimeningococcic serum. Blood was taken for

agglutination tests at 10.30 a.m., 1.15, 5.15, and 9.30 p.m. Lumbar puncture at 10.50 a.m., 1.25, 5.45, and 9.45 p.m. May 15. Bleedings at 10.45 a.m. and 4.18 p.m. Lumbar puncture at 10.50 a.m. and 4.23 p.m. The samples were tested May 16 for agglutinins with a parameningococcus, and the results are recorded in Text-fig. 1, *a*.




TEXT-FIG. 1, *a*, *b*, and *c*. (*a*) Meningococcic agglutinins in the blood and spinal fluid of the monkey after intraspinal injection of 3 cc. of antimeningococcic serum. (*b*) Meningococcic agglutinins in the blood and spinal fluid of the monkey after intravenous injection of antimeningococcic serum and the intraspinal injection of normal horse serum. (*c*) Meningococcic agglutinins in the blood and spinal fluid of the monkey after combined intravenous and intraspinal injection of antimeningococcic serum.

*Macacus rhesus* H. May 13, 1918, 5 p.m. Injected intraspinally 2 cc. of normal horse serum. May 14, 8.10 a.m. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. Specimens of spinal fluid and blood were taken at 9.10 a.m., 12.10 and 8.10 p.m., and at 9.10 a.m. on May 15. The results of the agglutination tests with parameningococcus on May 16 are recorded in Text-fig. 1, *b*.

*Macacus rhesus* I. May 14, 1918, 9.30 a.m. Injected intraspinally 3 cc. and intravenously 20 cc. of polyvalent antimeningococcic serum. Samples of blood and spinal fluid were removed at 11 a.m., 1.30, 6, and 9.40 p.m. May 15. Specimens taken at 10.50 a.m. and 4.25 p.m. The results of agglutination tests with parameningococcus on May 16 are shown in Text-fig. 1, *c*.

## CONCLUSIONS.

Agglutinins for the meningococcus were not found in the spinal fluid of normal monkeys which had received antimeningococcic serum intravenously.

The intraspinal injection of isotonic salt solution, normal horse serum, or a culture of living meningococci allows agglutinins for the meningococcus to pass from the blood to the spinal fluid of the passively immunized monkey; and the rate of the passage is affected by the severity of the inflammation induced in the meninges.

The rates of elimination from the blood and spinal canal of meningococcic antibodies, as shown by the agglutination reaction, were compared in monkeys treated with immune serum (*a*) intraspinally, (*b*) intravenously, and (*c*) intraspinally and intravenously in combination.

(*a*) When immune serum is given intraspinally the agglutinins are very much diminished after 8 hours and practically disappear at 12 hours. They appear in the blood at the 4th hour after injection and quickly diminish.

(*b*) After intravenous injection of immune serum, when the meninges are inflamed, agglutinins appear in the spinal fluid in small amounts in about 12 hours and increase to the 25th hour. More than one-half of the agglutinins disappear from the blood within 8 hours and remain in low concentration at 25 hours.

(*c*) After combined intraspinal and intravenous injection the agglutinins remain in higher concentration in the spinal fluid and for a longer time than by method (*a*) or (*b*). The curve descends after 12 hours, and agglutinins are present at 25 hours. They remain in maximum concentration in the blood for 25 hours.

# EXPERIMENTS ON THE MODE OF INFECTION IN EPIDEMIC MENINGITIS.

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Renewed interest has been aroused in the manner in which the meningococcus reaches the meninges from the upper respiratory mucous membrane. That the portal of entry of the meningococcus into the body is the mucosa of the nasopharynx is generally admitted. The point which has not been settled is whether the meningeal invasion is lymphatic or hematogenous. The most direct route would be along the lymphatics of the olfactory filaments to the meninges of the base of the brain; but no convincing evidence has been brought forward for that mode of invasion.

Westenhoeffer[1] conceived the idea that the meninges became infected from the sphenoidal sinuses; but later he abandoned that view.[2] Flexner[3] noted that when meningococci were injected intraspinally into monkeys by lumbar puncture film preparations of the nasal mucosa showed a small number of polymorphonuclear leucocytes believed to be derived from the inflamed meninges, containing Gram-negative diplococci resembling meningococci. He failed, however, to recover meningococci in cultures from the nasal mucosa of the inoculated animals, but he nevertheless discussed the possibility of a reverse process by which the meningococcus might be carried through lymphatics to the meninges in man.[4] This possibility was strengthened by the later observations on experimental poliomyelitis. The virus of poliomyelitis differs from the meningococcus in having a far wider range of pathogenicity for the monkey. While the meningococcus must be injected in considerable amounts directly into the subarachnoid space in monkeys in order to incite a fatal meningitis[3] and is entirely ineffective when

[1] Westenhoeffer, *Berl. klin. Woch.*, 1905, xlii, 737.

[2] Westenhoeffer, M., *Berl. klin. Woch.*, 1906, xliii, 1267, 1313.

[3] Flexner, S., *J. Exp. Med.*, 1907, ix, 142.

[4] The recovery of the organism was later accomplished in these laboratories from the nasal mucosa of monkeys which had received injections of the living meningococci intraspinally.

applied to the nasal mucosa, the application of small quantities of the virus of poliomyelitis to the latter is often followed by paralysis;[5] on the other hand, large doses of the virus often fail to induce infection when injected directly into the blood.[6]

Up to the present no satisfactory solution of the problem of the mode of infection in epidemic meningitis has been secured, and no considerable additions to our knowledge of the subject have been made since the pandemic of 1905–10. The appearance of the disease among the Western Armies has again stimulated interest in the problem. Naturally enough this interest has been primarily derived from clinical observations, and efforts at a better therapeutic control of cases. The newer observations indicate that the meningococcus is more frequently present in the circulating blood than has hitherto been supposed, and, especially, that a condition of hematogenous infection with the meningococcus may precede[7] any demonstrable infection or inflammation of the meninges, and, finally, that it is less rare than has been believed for a general infection with the meningococcus to exist independently of meningeal involvement.[8] The changing therapeutic point of view brought about by

[5] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 45.

[6] Clark, P. F., Fraser, F. R., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 223.

[7] Handa, H., and Nanjo, M., *Centr. Bakteriol., Ref.*, 1914, lxi, 82. Bonnel and Joltrain, E., *Bull. et mém. Soc. méd. Hôp. Paris*, 1916, xl, 75.

[8] Andrewes, F. W., *Lancet*, 1906, i, 1172. Liebermeister, G., *Münch. med. Woch.*, 1908, lv, 1978. Elser, W. J., and Huntoon, F. M., *J. Med. Research*, 1909, xx, 371. Bovaird, D., *Arch. Int. Med.*, 1909, iii, 267. Monziols and Loiseleur, *Bull. et mém. Soc. méd. Hôp. Paris*, 1910, xxix, 155. Chevrel, F., and Bourdinière, J., *Bull. et mém. Soc. méd. Hôp. Paris*, 1910, xxx, 165. Cecil, R. L., and Soper, W. B., *Arch. Int. Med.*, 1911, viii, 1. Barral, Coulomb, and Couton, *Bull. et mém. Soc. méd. Hôp. Paris*, 1912, xxxiii, 829. Bray, H. A., *Arch. Int. Med.*, 1915, xvi, 487. Sainton, P., and Maille, J., *Bull. et mém. Soc. méd. Hôp. Paris*, 1915, xxxix, 296, 374. Elliott, W. M., *Lancet*, 1916, ii, 1010. Pybus, F. C., *Lancet*, 1917, i, 803. Netter, A., Salanier, M., and Wolfrom, *Compt. rend. Soc. biol.*, 1916, lxxix, 973. Worster-Drought, C., and Kennedy, A. M., *Lancet*, 1917, ii, 711. Symmers, W. S., *Brit. Med. J.*, 1917, ii, 789. Anderson, W., McNee, J. W., Brown, H. R., Renshaw, A., McDonnell, J., Davidson, F. C., Gray, A. C. H., and Herringham, W. P., *J. Roy. Army Med. Corps*, 1917, xxix, 473. Sainton, P., *Paris méd.*, 1918, viii, 86. Zeissler, J., and Riedel, F., *Deutsch. med. Woch.*, 1917, xliii, 258. Thomsen, O., and Wulff, F., *Hospitalstid.*,

these experiences has culminated in the observations just published by Herrick[9] and others.[8]

The fact, now fairly established by studies carried out very early in cases of epidemic meningitis among our recruits, that a meningococcemia may precede an actual demonstrable meningococcus invasion of the subarachnoid space, has brought up the question whether the condition favored or hindered the development under these conditions of the meningitis itself. In civil practice, where cases are not observed so early in the attack, it is exceptional to have to deal with symptoms referable to irritation of the meninges but in which meningococci are present in the blood or even in the cerebrospinal fluid, without, however, being attended by cellular or chemical changes in the cerebrospinal fluid itself. In other words, under the latter circumstances the indications of inflammation are usually present in the meninges, calling for the intraspinal injection of serum. When, however, it happens that no evidence of inflammation is revealed by the lumbar puncture, the question has arisen as to the advisability of making any intraspinal injections of the serum whatsoever.

The reasons for doubt in this instance are two: first, whether any therapeutic benefit will follow; and second, and more important, whether the aseptic meningitis set up by the antimeningococcic serum may favor the local inflammatory attack of the meningococci. The latter point has been raised by Herrick because of the experiments of Flexner and Amoss[10] on the promoting action of an aseptic meningitis induced by serum and other agents on infection in poliomyelitis; but it disregards the important consideration that such an aseptic inflammation is only favoring when a non-immune serum is employed. An immune antipoliomyelitic serum equally sets up an aseptic inflammation, but it also prevents the infection's arising.[10,11] There is therefore no exact analogy to be found be-

1917, lx, 1192, abstracted in *J. Am. Med. Assn.*, 1918, lxx, 498. Baeslack, F. W., Bunce, A. H., Brunelle, G. C., Fleming, J. S., Klugh, G. F., McLean, E. H., and Salomon, A. V., *J. Am. Med. Assn.*, 1918, lxx, 684. Baron and Dumont, *Presse méd.*, 1917, xxv, 394.

[9] Herrick, W. W., *Arch. Int. Med.*, 1918, xxi, 541.

[10] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

[11] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 525.

tween these experiments on poliomyelitis and the hypothetic problem referred to in meningococcus infection in man.

On the other hand, the newer knowledge on meningococcus infection suggests that experiments be undertaken to determine whether an aseptic meningitis may not promote the passage of meningococci from the blood into the meninges and thus contribute to the inciting of meningococcus inflammation. The monkey is the most suitable animal for these experiments.

In the first place, the monkey can be given meningitis by an intraspinal injection of a suitable culture of the meningococcus. The infection in this case runs a mild or fatal course, depending on the virulence and dose of the culture employed. From the meninges meningococci escape into the general circulation but do not multiply and are quickly suppressed there. When the cultures are injected directly into the blood they do not appear in the meninges in normal monkeys but tend soon to disappear; a meningitis does not arise under these circumstances.

The preceding considerations led us to devise a series of experiments to determine the influence of an aseptic meningitis on the intravenous inoculation of cultures of virulent meningococcus in monkeys. The aseptic inflammation was incited by (*a*) normal horse serum, (*b*) normal salt solution, and (*c*) protargol. The last chemical not only induces an inflammation, but it paralyzes phagocytosis as well, and because of that action intensifies the local action of the meningococcus.[12]

## EXPERIMENTAL.

The first experiment was arranged to determine the fact of the survival of the meningococcus in the blood and whether it would pass into the cerebrospinal fluid of a normal monkey. Meningococci vary greatly, as stated, in their initial power to set up a severe or fatal meningitis in monkeys on intraspinal injection.[3] When the effect is too slight the virulence of some cultures can be heightened by passage through monkeys or guinea pigs. All monkeys used were *Macacus rhesus*.

[12] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1916, xxiii, 683.

*Intraspinal Inoculation.*—An irregular strain of the meningococcus, tending toward the para type, was passed through two monkeys by intraspinal injection, as indicated below, when it acquired a suitable degree of virulence for use. A sheep serum agar slant of a growth 14 hours old was suspended in 2 cc. of isotonic salt solution. Mar. 14, 1918, 4 p.m. Monkey 1 received the entire suspension intraspinally, by lumbar puncture. Mar. 15, 10 a.m. Animal ill. Lumbar puncture, 1 cc. of cloudy fluid containing polymorphonuclear cells and many meningococci. The cocci were mostly extracellular and of typical morphology. A blood culture taken at the same time was negative. Mar. 16, 11 a.m. Animal very ill. Lumbar puncture, 1 cc. of turbid fluid. Microscopic examination showed many pus cells and numerous Gram-negative diplococci, many phagocyted. Two drops of the fluid on sheep serum agar yielded abundant growth. The animal may or may not have recovered, but as the purpose was to recover the meningococcus for subsequent inoculation it was given 2 cc. of antimeningococcic serum intraspinally. Recovery followed.

The culture obtained on Mar. 15 from the spinal fluid was transferred on Mar. 17 to a tube of sheep serum dextrose agar and the growth suspended in the manner described and injected intraspinally at 11.45 on Mar. 18 into Monkey 2. 5.15 p.m. Animal ill. Temperature 39.2°C. Lumbar puncture, 1.5 cc. of turbid fluid under pressure. Microscopically there were large numbers of meningococci and pus cells; few cocci had been phagocyted. The culture yielded abundant growth. Blood culture positive. As the purpose again was to recover the culture, 2 cc. of antimeningococcic serum were injected. The animal gradually recovered.

A third suspension of this meningococcus was prepared in the manner described and injected intraspinally into Monkey 3 on Mar. 20 at 12.10 p.m. 6 hours later the monkey was very ill. Lumbar puncture was performed and antimeningococcic serum administered. Blood culture positive. 3 cc. of turbid fluid were recovered by the lumbar puncture. It contained large numbers of pus cells and meningococci, none intracellular. Mar. 21. Lively. Mar. 22. Completely recovered.

It is obvious from the brief descriptions given that the culture of meningococcus quickly rose in virulence and set up severe symptoms in shorter periods. The following experiments were made with this potent culture.

*Experiment 1. Intravenous Inoculation.*—Mar. 22, 1918, 11 a.m. *Macacus rhesus* given one-half of a suspension of 18 hour growth of culture of meningococcus obtained from Monkey 3 intravenously. 5 p.m. No symptoms. 5 cc. of blood withdrawn from vein. 3 cc. laked and plated. 2 cc. planted in dextrose broth. Former remained sterile; latter gave growth of meningococci.

Lumbar puncture yielded clear fluid devoid of cocci or cells. Mar. 24. No symptoms. Lumbar puncture, no fluid. No symptoms subsequently developed. Apr. 1. Animal lively. Apr. 2. Found dead.

*Autopsy.*—No visceral changes; no cause of death established. Microscopic examination of the central nervous system showed no lesions of any kind. The cause of death can only be conjectured. Possibly it was due to delayed endotoxin intoxication.

This experiment confirms many previously made which are to the effect that an intravenous injection of meningococci into monkeys followed by several lumbar punctures does not set up a meningitis even when the culture is active on meningeal inoculation.

In the next experiments the effects of aseptic meningitis were investigated. In these a strain of parameningococcus recently cultivated from the blood of a case of meningitis in man was employed. It was first tested by intraspinal injection into a *Macacus rhesus* and proved virulent.

*Experiment 2. Intravenous Inoculation Following Intraspinal Horse Serum Meningitis.*—Mar. 24, 1918, 4 p.m. *Macacus rhesus* received 2 cc. of normal horse serum intraspinally. Mar. 25, 10 a.m. Suspension of one-half of sheep serum dextrose agar slant culture in 10 cc. of isotonic salt solution injected intravenously. 5 p.m. 0.5 cc. of blood withdrawn from vein and placed in dextrose broth yielded a growth of meningococcus. Mar. 26, 10 a.m. Lumbar puncture, 0.5 cc. of slightly turbid fluid containing 400 polymorphonuclear cells per c.mm. No meningococci on stained films prepared from centrifuged sediment or in cultures. 5 p.m. same day. 5 cc. of blood taken, laked by addition of two parts of sterile distilled water, centrifuged, and residue smeared on plates. Innumerable colonies of meningococci appeared on the plates. 72 hours after the intravenous inoculation the animal was well; a blood culture was negative, and 0.5 cc. of clear fluid obtained by lumbar puncture was free of meningococci by microscopic and culture test. The monkey remained well.

This experiment was repeated with the same result. The interval between the intraspinal injections of horse serum and the intravenous inoculations of the culture was, however, 40 hours. 1 cc. of blood withdrawn 96 hours after the intravenous injection in dextrose broth yielded a pure culture of meningococcus.

Reference has already been made to the promoting action of an intraspinal injection of normal saline solution in poliomyelitis. As the salt solution sets up an aseptic meningitis of milder degree than

serum it was used in the experiments with meningococcus merely to exclude any protective local action of large numbers of polymorphonuclear leucocytes such as are brought into the meninges by horse serum.

*Experiment 3. Intravenous Inoculation Following Intraspinal Saline Solution.*—The culture of parameningococcus had been passed through two monkeys and recovered from the circulating blood. Apr. 23, 1918, 4 p.m. *Macacus rhesus* received 2 cc. of isotonic salt solution. Apr. 25, 10 a.m. (42 hours after intraspinal injection). Injected intravenously one-half of a 16 hour growth of meningococcus on a sheep serum dextrose agar slant in 10 cc. of isotonic saline solution. Apr. 26, 10 a.m. Lumbar puncture, 1 cc. of clear fluid obtained. Film preparations and culture from bottom of tube after long centrifugation revealed no meningococci. Blood culture of 5 cc. in blood dextrose broth yielded heavy growth of meningococci. Apr. 27, 10 a.m. 5 cc. of blood withdrawn for culture in dextrose broth yielded meningococci. Lumbar puncture, 2 cc. of slightly turbid fluid which was centrifuged at high speed. Film preparations showed polymorphonuclear leucocytes and few round cells but no meningococci. 1 cc. of the fluid containing the sediment planted in dextrose blood broth showed no growth. Apr. 29, 10 a.m. Monkey active and lively. Lumbar puncture, 0.5 cc. of slightly turbid fluid. No meningococci were found on microscopic examination and culture. Blood culture, 5 cc. in dextrose broth negative.

Protargol injected intraspinally not only sets up an inflammation of the meninges, but is antiphagocytic in its action against meningococci. Hence it was employed to promote an intraspinal infection by way of the blood stream.

*Experiment 4. Intravenous Inoculation Following Intraspinal Protargol.*—Mar. 21, 1918, 4 p.m. *Macacus rhesus* received intraspinally 2 cc. of a 0.5 per cent aqueous dilution of protargol. Mar. 22, 10.30 a.m. Injected intravenously one-half of a 16 hour slant culture on sheep serum dextrose agar of the virulent parameningococcus in 10 cc. of isotonic salt solution. 5 p.m. Withdrew 5 cc. of blood, which, after laking with sterile distilled water and centrifuging, were plated on blood agar and yielded a confluent growth of meningococcus. Mar. 23, 10 a.m. Blood culture, 5 cc. plated as before yielded about 200 colonies of meningococci. Lumbar puncture, withdrew 0.5 cc. of slightly turbid fluid. Microscopic examination of film preparation showed a few fragmented, poorly staining polymorphonuclear cells and debris but no meningococci. Culture in blood broth was negative. Mar. 24. Found dead.

*Autopsy.*—Some congestion of brain at the base. Cultures and film preparations from meninges, cortex, lateral ventricles, and base showed no meningococci or other microorganisms. Microscopic sections showed slight meningeal inflammation as indicated by a collection of leucocytes probably due to the protargol injection.

This experiment was repeated so that the observations extended over a somewhat longer period of time. Because of the early disappearance of the meningococci from the blood after injection several intravenous injections of living meningococci were made at intervals to prolong the period of the meningococcemia.

*Experiment 5. Multiple Intravenous Inoculations Following Intraspinal Injection of Protargol.*—Mar. 27, 1918, 4 p.m. *Macacus rhesus* injected intraspinally with 2 cc. of a 0.5 per cent aqueous dilution of protargol. Mar. 28, 11 a.m. Injected intravenously with one-half of a 16 hour growth on sheep serum agar of a virulent parameningococcus. 3 p.m. Blood culture positive; total leucocytes 10,200. Mar. 29, 11 a.m. Blood culture positive; lumbar puncture yielded 2 cc. of slightly turbid fluid containing fragmented cells but no meningococci. Cultures remained sterile. Mar. 30, 11 a.m. Lumbar puncture yielded 2 cc. of fluid; no meningococci found on culture or microscopic examination. Blood culture (5 cc.) positive; total leucocytes 7,600. Mar. 31, 11 a.m. Lumbar puncture, 0.5 cc. of fluid; no meningococci found by culture or microscopic examination. Blood culture (5 cc.) positive. Apr. 1, 11 a.m. Lumbar puncture, 3 cc. of fluid. No meningococci found by culture or microscopic examination. Blood culture (8 cc. in broth) negative.

The animal never showed any symptoms of meningitis and was allowed to rest 16 days to develop a partial immunity, for two purposes: first, to ascertain whether subsequent injections under conditions of partial immunity would allow meningeal invasion by the clumping of the organisms (*in vivo* agglutination) as sometimes happens in dogs injected with living pneumococci;[13] and second, to ascertain whether the organisms introduced by spinal injection would be found in the blood stream in the highly active immune animal.

Apr. 13. Injected intravenously one-half of a 20 hour growth of parameningococcus on slant serum agar in 10 cc. of isotonic salt solution. Apr. 20. Injected intravenously three-quarters of a 16 hour culture of parameningococcus. Apr. 27. Injected the entire culture. The animal remained well. Before injection serum agglutinated the homologous parameningococcus completely in a dilution of 1:1,200 and other parameningococci in 1:1,000. May 2, 5 p.m. Injected intraspinally in 5 cc. of isotonic salt solution entire 8 hour growth of virulent parameningococci on slant serum agar. May 3, 11 a.m. Total white blood cells 24,200. 5 cc. of blood withdrawn for culture yielded meningococci. Lumbar puncture, 0.1 cc. of slightly turbid fluid from which large numbers of meningococci were grown. May 4, 11 a.m. Growth obtained from 1.5 cc. of blood withdrawn for culture. Total white blood cells 40,400. Lumbar puncture, 2 cc. of turbid fluid from which cultures were positive. Microscopic exami-

[13] Bull, C. G., *J. Exp. Med.*, 1916, xxiv, 7.

nation showed numerous Gram-negative diplococci intracellular; few extracellular. May 5. Total white blood cells 12,400. Blood culture (5 cc.) negative. Lumbar puncture, 3 cc. of slightly turbid fluid containing many phagocyted Gram-negative diplococci, none extracellular. Cultures negative. May 6. Total white blood cells 12,000. Blood culture (5 cc.) negative. Lumbar puncture, 3 cc. of slightly turbid fluid. Culture negative. No organisms found on microscopic examination.

In this experiment the living virulent meningococci circulated in the blood for at least 72 hours during an aseptic inflammation of the meninges without passing through and causing meningitis. The monkey was actively immunized by intravenous injections so that the serum agglutinated the meningococcus in a dilution of 1:1,200. The intraspinal injection of meningococci was followed by meningococcemia lasting 48 hours, showing that whereas the passage of these organisms from the blood to the cerebrospinal fluid could not be brought about even under conditions promoting interchange, ready passage in the opposite direction from the meninges to the blood was accomplished when antibodies were circulating in the blood.

*Experiments with Rabbits.*

Austrian[14] reports that he was able to observe localization of the meningococcus in the meninges and typical fatal meningitis after intravenous injection in three rabbits out of twenty which had received a previous intraspinal injection of normal rabbit serum. In the rabbits which received no preparatory intraspinal injections localization did not occur.

However, it is stated in his typical protocol that after the intraspinal injection of normal rabbit serum "injury of the cord caused twitching of the tail and spastic palsy of the left hind leg, and dyspnœa, coma and ataxia developed." It is possible, therefore, that mechanical rather than chemical injury made possible the passage of the microorganisms from the blood to the spinal fluid. In order to test the validity of this view two series of rabbits were injected intraspinally with normal rabbit serum. In the first series great care was taken to avoid mechanical injury to the cord, while in the second series

[14] Austrian, C. R., *Bull. Johns Hopkins Hosp.*, 1918, xxix, 183.

the needle was introduced so that spastic paralysis followed the procedure. The animals of both series subsequently received intravenous injections of living meningococci.

*Series I.*—Five rabbits weighing about 2 kilos received intraspinally 1.5 cc. of normal rabbit serum. Half an hour later there were injected intravenously in 10 cc. of saline solution the growths from two 16 hour cultures on slant serum agar of a virulent irregular meningococcus. Rabbit 1 was etherized 4 hours later, No. 2 died in 6 hours, No. 3 was etherized at 8 hours, and Nos. 4 and 5 at 24 hours. Cultures and film preparations from the cord, cortex, pons, base of brain, and heart's blood showed no meningococci.

*Series II.*—Rabbit 6, weight 2.2 kilos, injected intraspinally with 1.5 cc. of normal rabbit serum on Oct. 15, 1918, 3 p.m. 3.05 p.m. Spastic; paralysis of both hind legs. Oct. 16, 10 a.m. No paralysis. Injected intravenously, in 10 cc. of isotonic salt solution, the 16 hour growth from two serum agar slants of a virulent irregular meningococcus. 4 p.m. No symptoms. Etherized and autopsied. Lumbar portion of cord hemorrhagic. Film preparations of cord and base of brain showed polymorphonuclear leucocytes and a few Gram-negative diplococci, none on cortex. Cultures from heart's blood and base of brain yielded irregular meningococci.

Rabbit 7, weight 2 kilos, received intraspinally 1.5 cc. of normal rabbit serum on Oct. 15, 1918, 3 p.m. 3.30 p.m. Spastic paralysis of left hind leg. Oct. 16, 10 a.m. Paralysis persisted. Injected intravenously in 10 cc. of isotonic salt solution 16 hour growth from two serum agar slants of a virulent irregular meningococcus. Oct. 17, 10 a.m. Paralysis persisted. No other symptoms. Etherized.

*Autopsy.*—Small hemorrhage in lumbar portion of cord. The blood vessels of the pia and dura were congested. Spinal fluid was turbid. Film preparations from spinal meninges, pons, and base of brain showed considerable number of polymorphonuclear leucocytes and many Gram-negative extracellular diplococci. Cultures from the spinal fluid, base of brain, and pons yielded meningococci, but the cultures from heart's blood were negative.

These experiments indicate that even under the conditions of congestion and chemical inflammation the meningococci do not pass from the blood to the spinal fluid in the rabbit unless a break in the continuity of the tissues occurs. The mechanical injury allows passage and localization of the meningococci. The results of the negative experiments in the monkey (Table I) correspond to those obtained in Series I with rabbits.

TABLE I.

*Results of Intravenous Injections into Monkeys of Living Virulent Meningococci Following Intraspinal Injections of Substances Producing Chemical Inflammation.*

| Experiment No. | Date. | Previous intraspinal injection. | Intravenous injection. | Last positive blood culture obtained at. | Result. |
|---|---|---|---|---|---|
| | *1918* | | | | |
| 1 | Mar. 22 | None. | One-half 16 hr. growth on serum agar slant. | 24th hr. | No localization in meninges. |
| 2 | " 25 | 2 cc. of normal horse serum. | " " | 24th " | " " |
| 3 | Apr. 24 | 2 cc. of normal horse serum. | " " | 96th " | " " |
| 4 | " 24 | 2 cc. of isotonic salt solution. | " " | 48th " | " " |
| 5 | Mar. 22 | 2 cc. of 0.5 per cent aqueous suspension of protargol. | " " | 24th " | " " |
| 6 | " 28 | 2 cc. of 0.5 per cent aqueous suspension of protargol. | " " | 72nd " | " " |

## DISCUSSION.

In discussing the several experiments described in this paper it will be well first to formulate the problem which presented itself for solution and then the means at hand to be employed for the purpose.

There has been no convincing finding either in man or in experimental animals to show how the meningococcus reaches the meninges. Two possibilities are obvious: lymphatic and hematogenous extension. The first would carry the meningococci directly from the nasopharynx to the base of the brain; the other indirectly, by way of the general circulation, to the subarachnoid space.

As long as cases of frank meningitis only came under observation the meningococci were usually found with ease in the turbid cerebrospinal fluid and possibly also in the blood. Flexner[3] showed in his experiments on the intraspinal inoculation of meningococci in monkeys that passage of the organism takes place from the subarachnoid space into the blood. Hence the mere finding of the

meningococci in the circulating blood in cases of meningitis in man could not be taken to indicate one way or the other their relation to the meningeal infection.

In one of the experiments recorded here (Experiment 5) intraspinal injection of meningococci into the actively immune monkey was followed by the appearance of these organisms in the blood, where they persisted for 48 hours. The serum at this time agglutinated the particular strain of meningococci in a dilution of 1: 1,200. This indicates that passage is far more easily accomplished by the meningococci in one direction, from the spinal fluid to the blood, than in the opposite direction, blood to spinal fluid.

The study of very early cases of infection in military establishments has thrown new light on the mechanism of meningococcus infection. For leaving aside the rare cases of meningococcemia without meningeal localization, it appeared that not infrequently cases would come under observation said to show no inflammatory changes in the meninges, as determined by a study of the spinal fluid, but with severe general symptoms of infection (including subcutaneous hemorrhages) in which meningococci were cultivable from the circulating blood. If these cases were not promptly treated and arrested by specific serum therapy, a meningitis is stated soon to develop. It may be that the presence of the meningococcus in the blood precedes their occurrence in the meninges or that the organism, on entering the cranial cavity and the blood simultaneously, is more readily recovered from the blood stream, or lastly that it is established in the upper portions of the central nervous system and overflows into the blood stream before evidences appear in the spinal fluid withdrawn at the remote and lower parts of the central nervous system.

In endeavoring to find a solution of the problem presented by experiments two points were kept in mind: first, that of a possible primary blood infection; and second, a promoting effect of the intraspinal injection of a serum on the localizations of the meningococcus in the meninges.

As regards the possibility of obtaining a decisive result by experiments on monkeys, the first point to be taken into account is the relative insusceptibility of the monkey to infection with the meningococcus. This degree of resistance is so great that Flexner[3] questions

whether, after an intraspinal injection, in which a fatal meningitis is set up, any actual multiplication of the microorganism takes place. He thinks it not impossible that from the beginning the injected micrococci are killed off, and the final fatal effect is caused by intoxication.

There can therefore be no very close analogy between the conditions experimentally induced and those existing in man when meningococcus infection arises. The experiments presented, however, are entirely clear on one point; namely, that in the monkey the meningococcus cannot be made to pass from the circulating blood, in which it is proved still to be surviving, to the aseptically inflamed meninges in such a way as to be detected there either by microscopic examination or in cultures. In other words, as far as the monkey is concerned the production of an aseptic meningitis is wholly without effect on the fate of meningococci injected into the blood stream.

The experiments are therefore in strong contrast to those made with the poliomyelitic virus, just as the great activity of the poliomyelitic virus when implanted on the nasal mucosa is in striking contrast to the innocuousness of the meningococcus applied to the same membrane. The experiments described in this paper emphasize, therefore, the unsuitability of the lower monkeys to solve the problem of the mode of meningococcus infections in man. And as far as they go they tend to show that an aseptic meningitis does not predispose the meninges to infection from the blood stream. Since in man the serum injected intraspinally is not normal but immune it can be presumed that the increased permeability of the meninges and choroid plexus induced by the chemical inflammation set up would be more than offset by the immunity principles introduced into the subarachnoid space along with the serum.

## CONCLUSIONS.

The lower monkeys as represented by *Macacus rhesus* are resistant to a high degree to infection with cultures of the meningococcus introduced into the general blood.

The lower monkeys are less resistant to infection when the meningococcus cultures are injected directly into the subarachnoid space by lumbar puncture.

Relatively virulent cultures, which have been passed through several monkeys, acquire the power of surviving in the circulating blood of the monkeys for a maximum period of about 72 hours. Nothing has, however, been observed to indicate that the injected meningococci actually multiply in the blood.

It has not been found possible to direct the meningococci circulating in the blood into the cerebrospinal meninges of monkeys. In this effort an aseptic meningitis was induced by injecting horse serum, saline solution, or protargol into the subarachnoid space preceding the introduction of the meningococci into the blood.

In rabbits the meningococci were able to pass into the spinal fluid from the blood when a physical break in the continuity was made; however, under the conditions of chemical inflammation of the meninges the rabbit reacted just as the monkeys, and the organisms did not pass.

Because of the high insusceptibility of the monkey to infection with meningococcus, it is not believed that the experiments throw any new light on the mode of invasion of the body in man by that microorganism.

The experiments do not lend any support to the notion that an intraspinal injection of the antimeningococcus serum, early in the course of invasion of meningococcus in man, and possibly at a period at which the meninges do not yet show evidences of inflammation, favors its diversion from the blood stream into the subarachnoid space.

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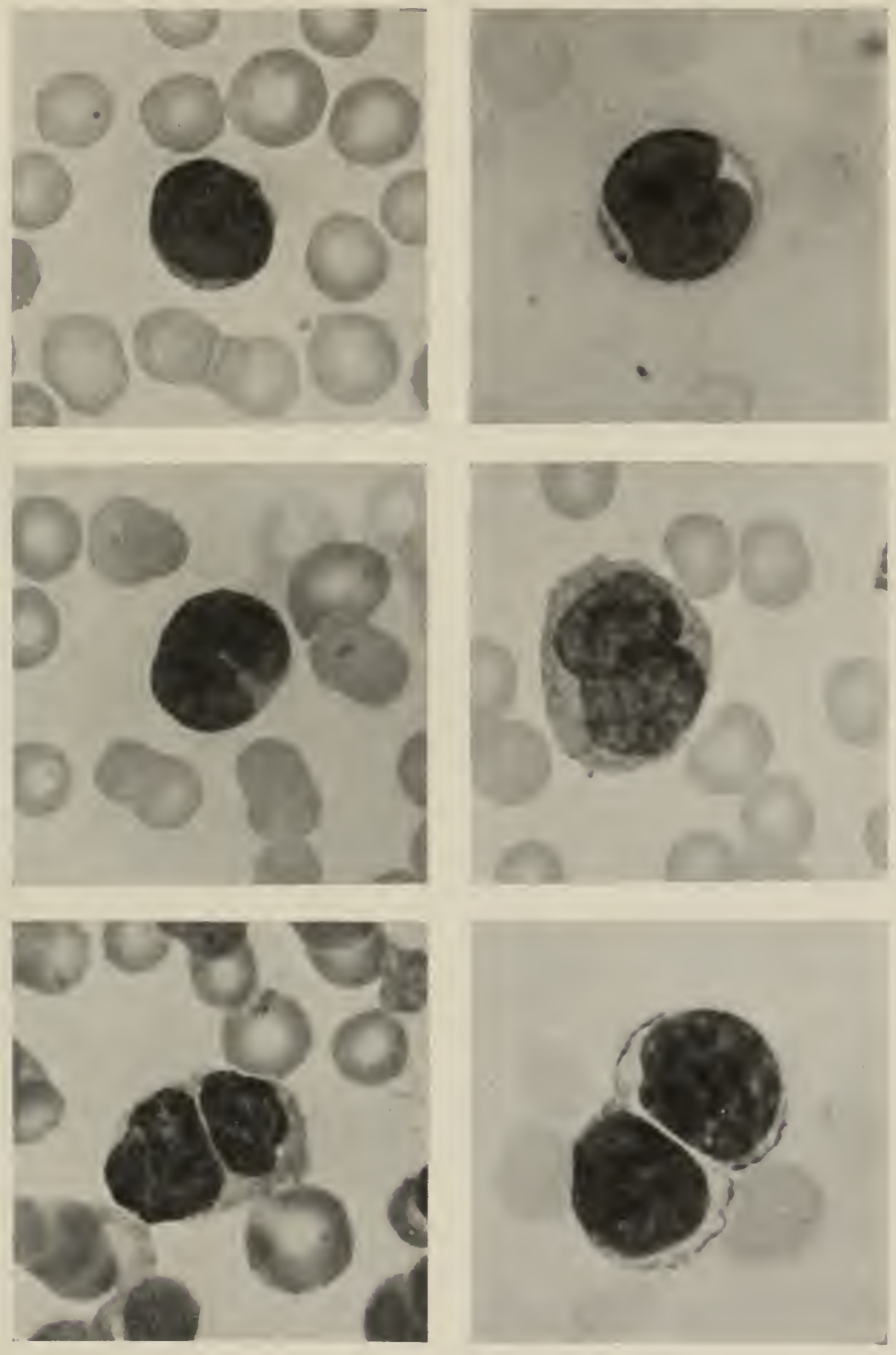


FIG. 1.

(Murphy and Sturm: Effect of dry heat on blood count. III.)

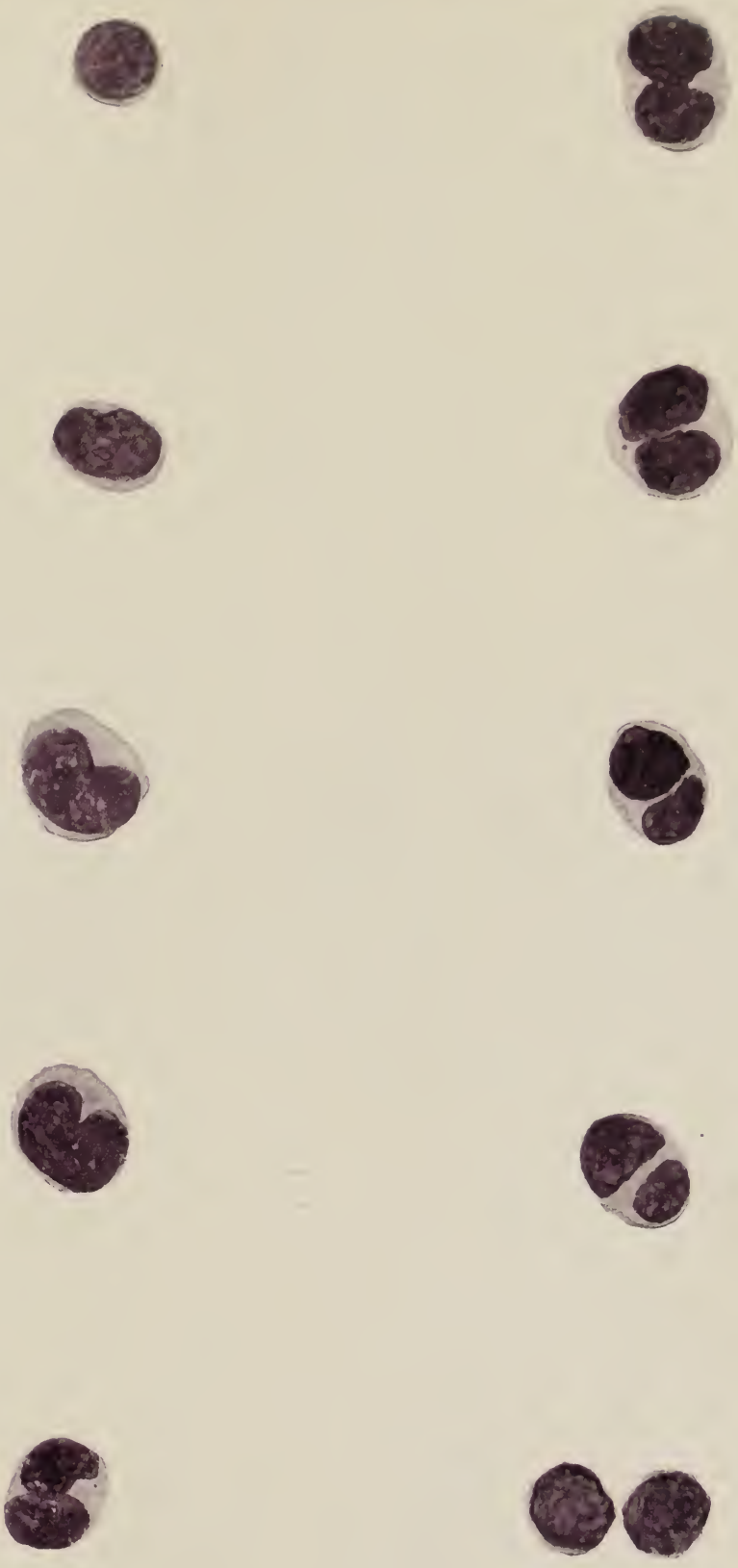


FIG. 2.

(Murphy and Sturm: Effect of dry heat on blood count. III)

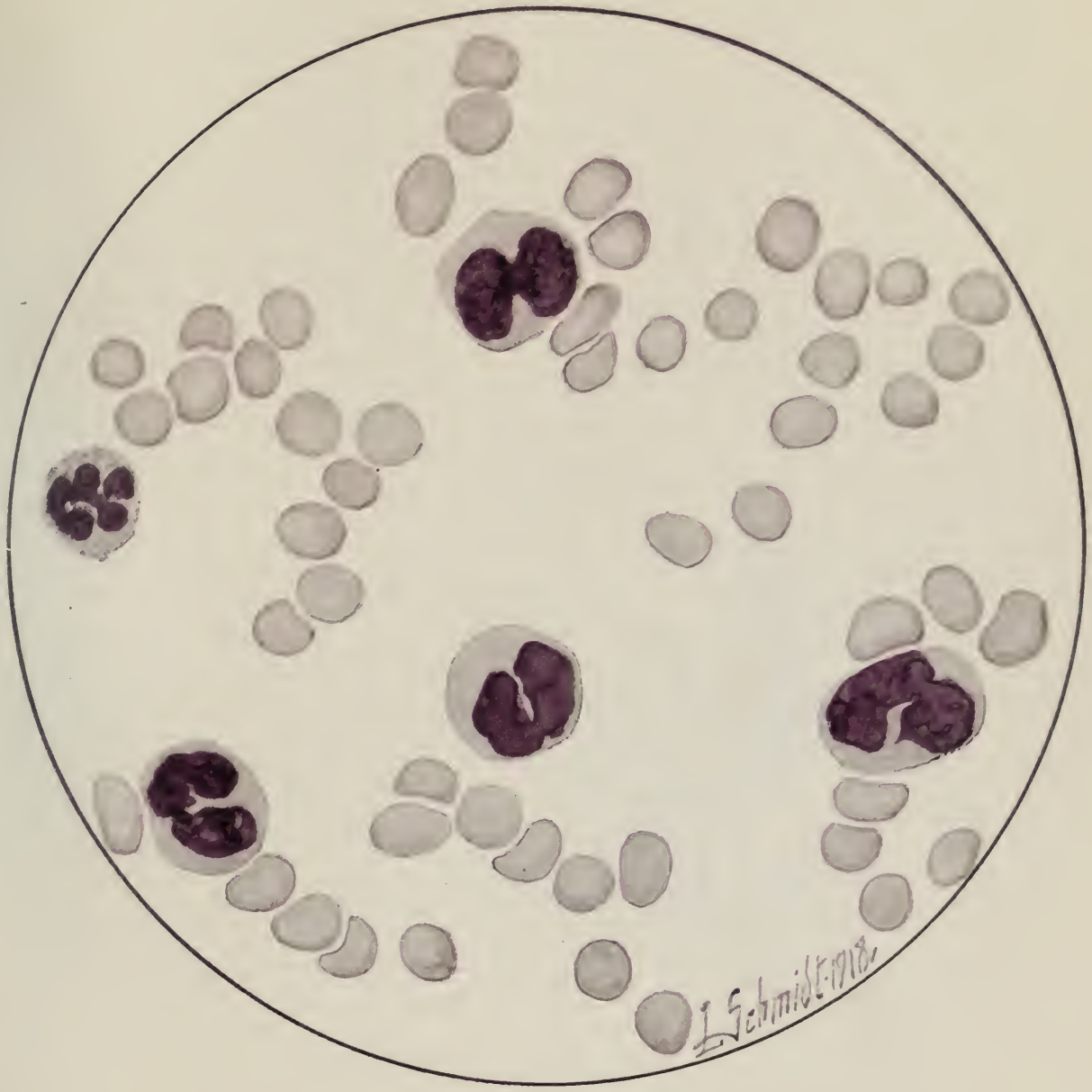
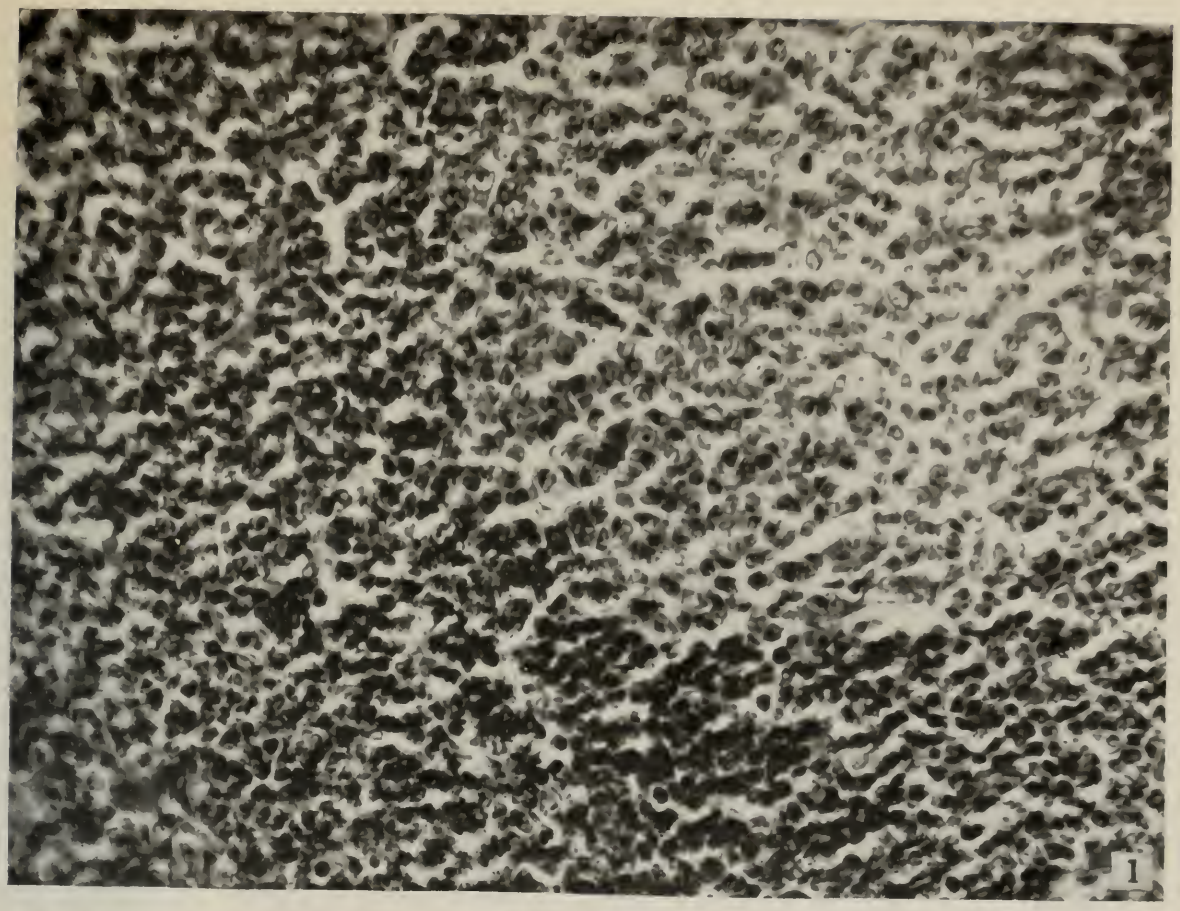
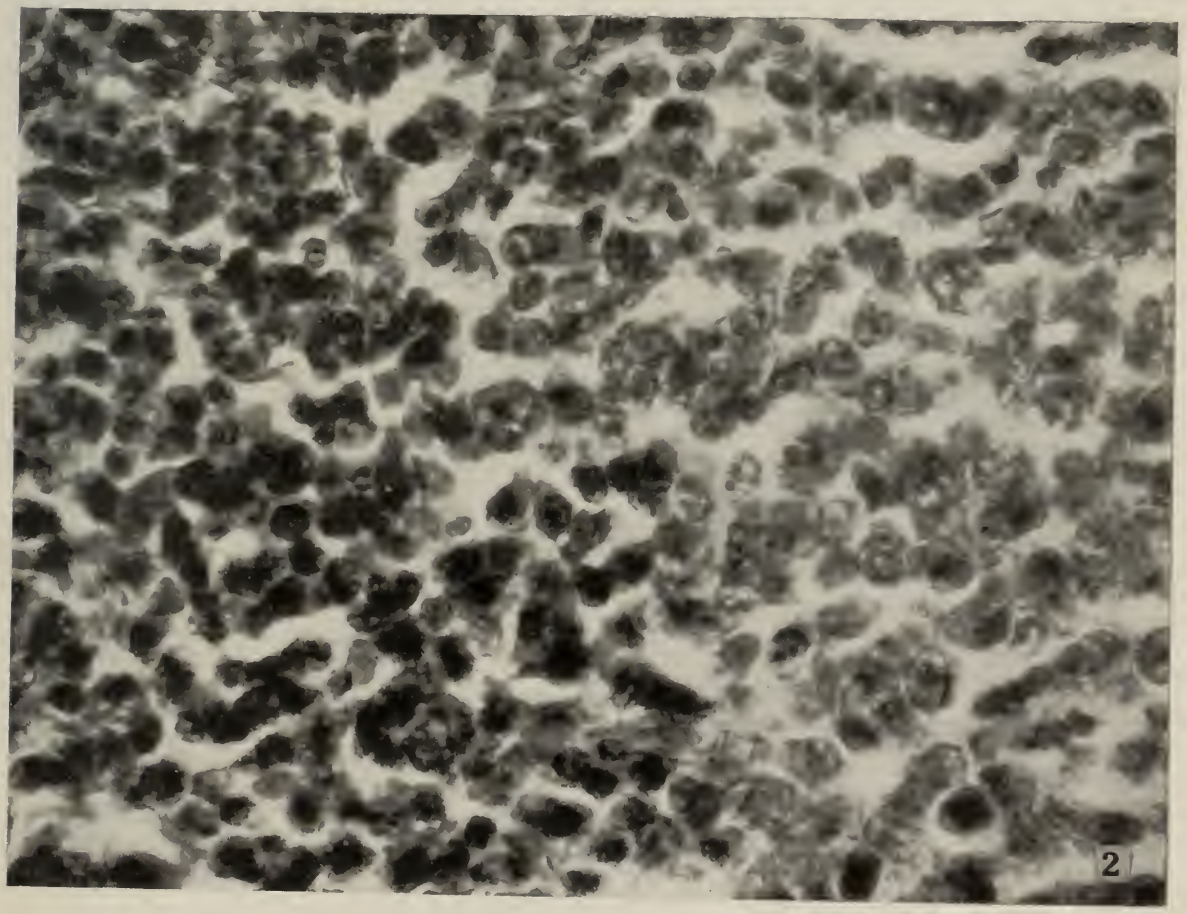


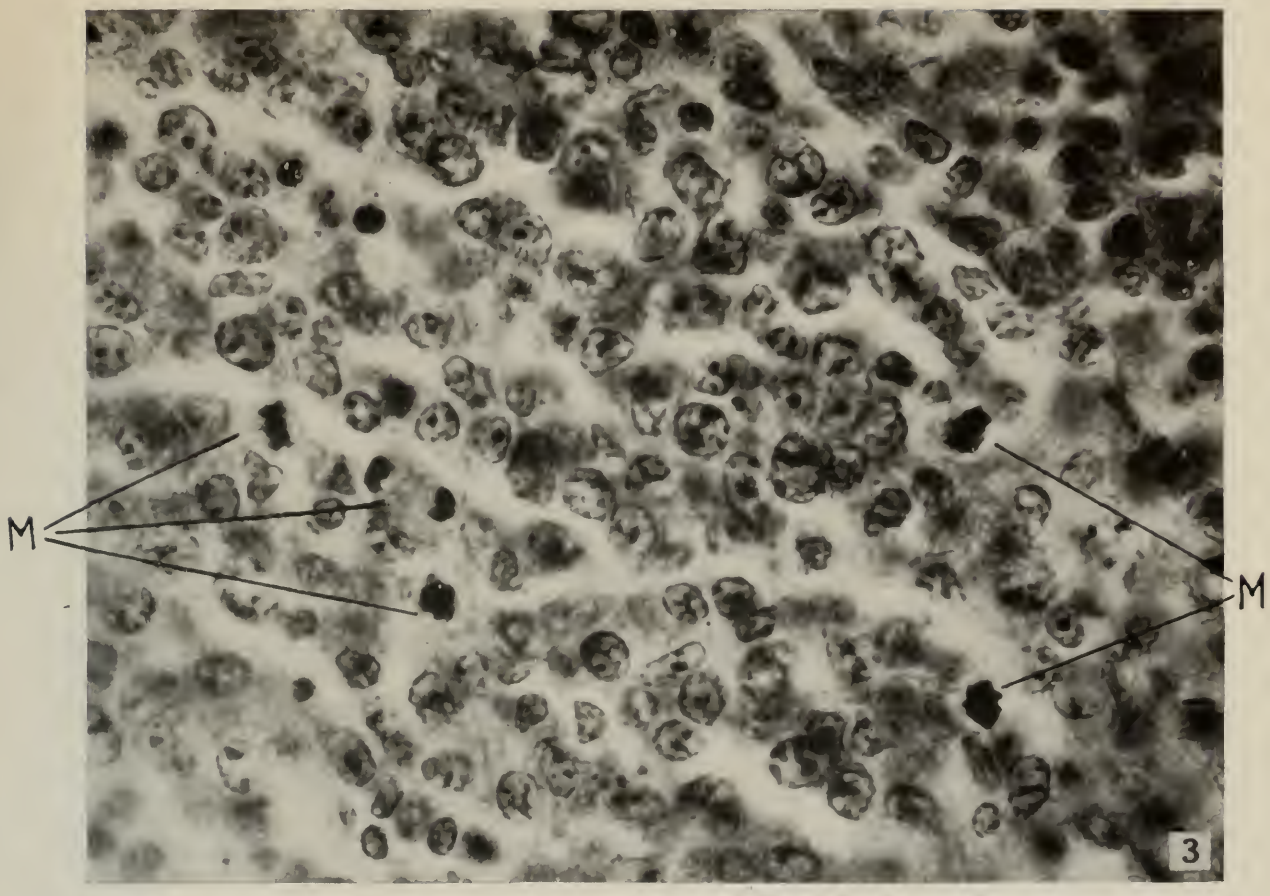

FIG. 3.

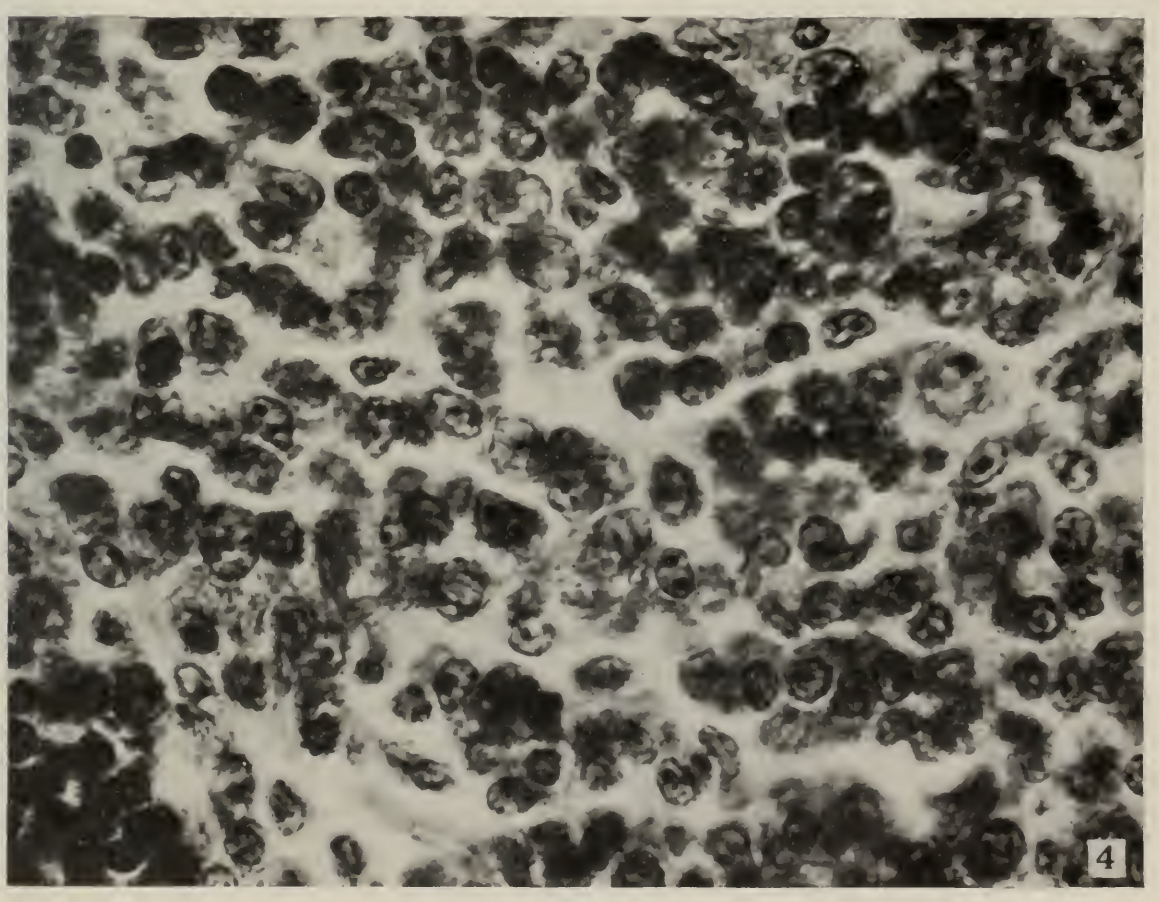
(Murphy and Sturm: Effect of dry heat on blood count. III.)



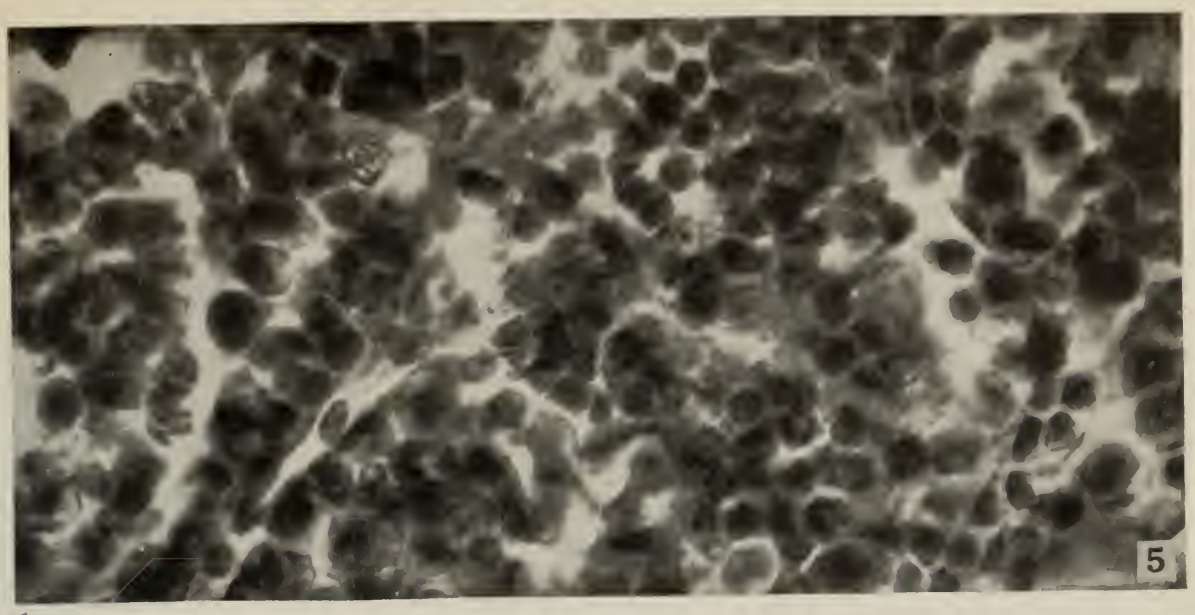



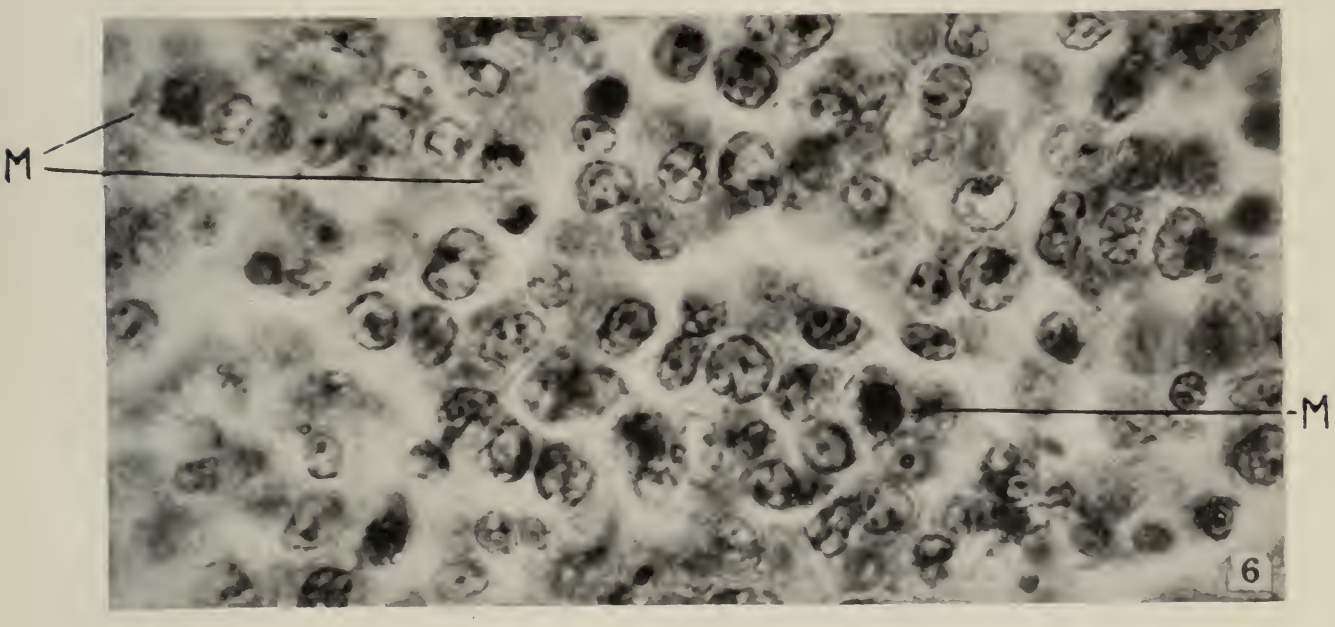

(Nakahara: Lymphocytosis induced by means of heat.)

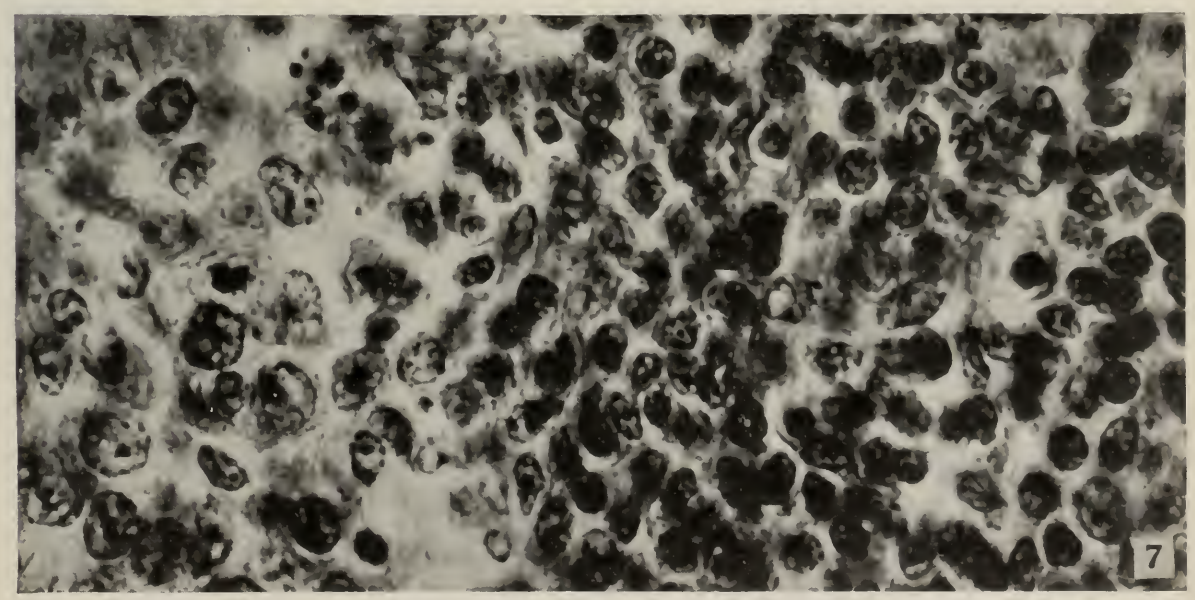


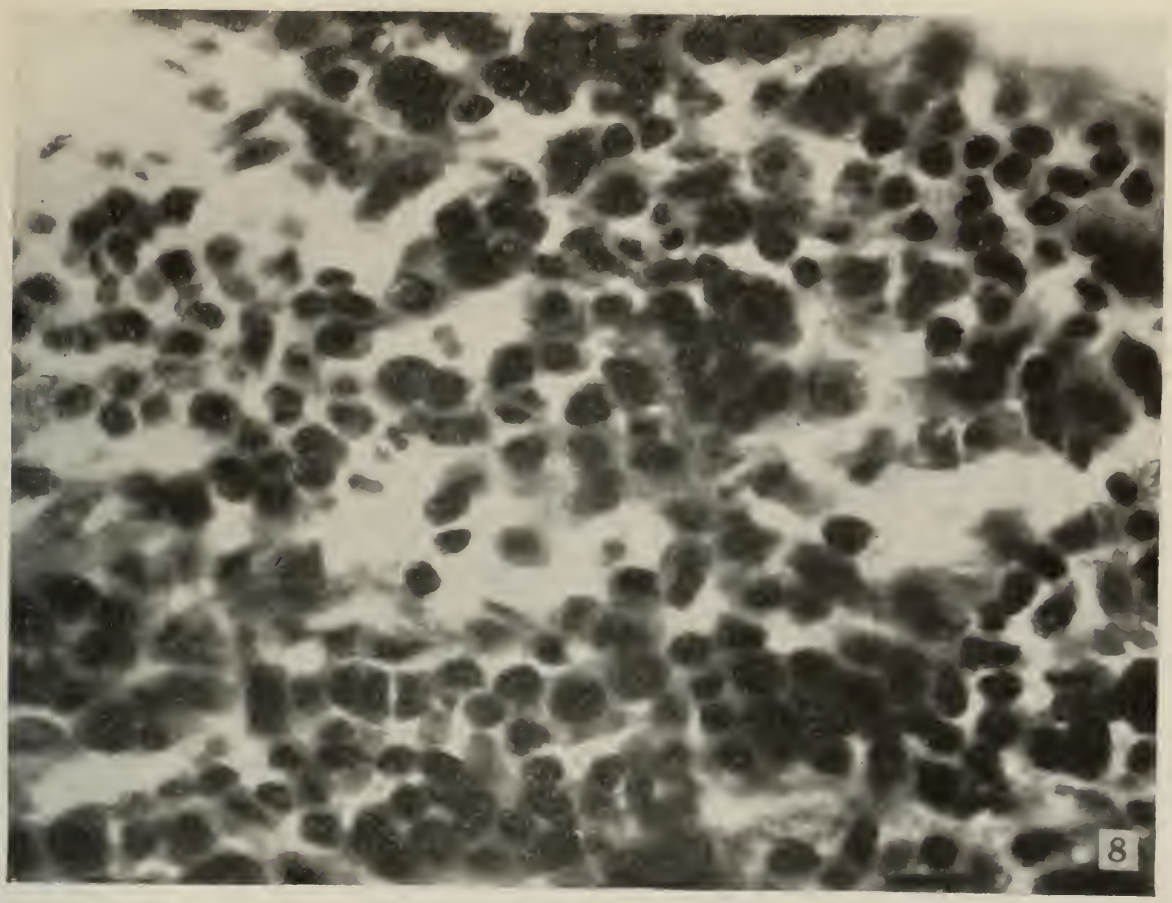

(Nakahara: Lymphocytosis induced by means of heat.)

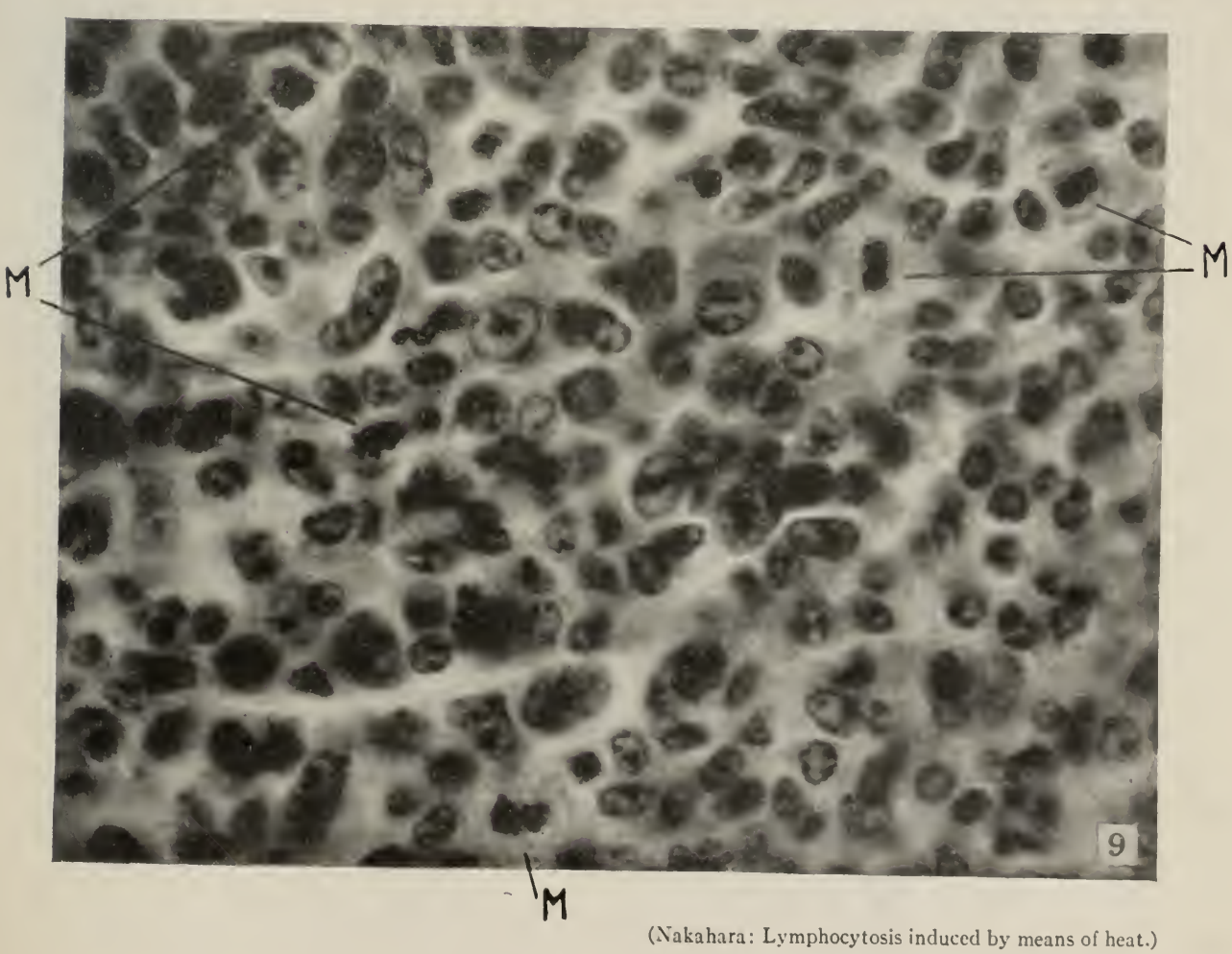


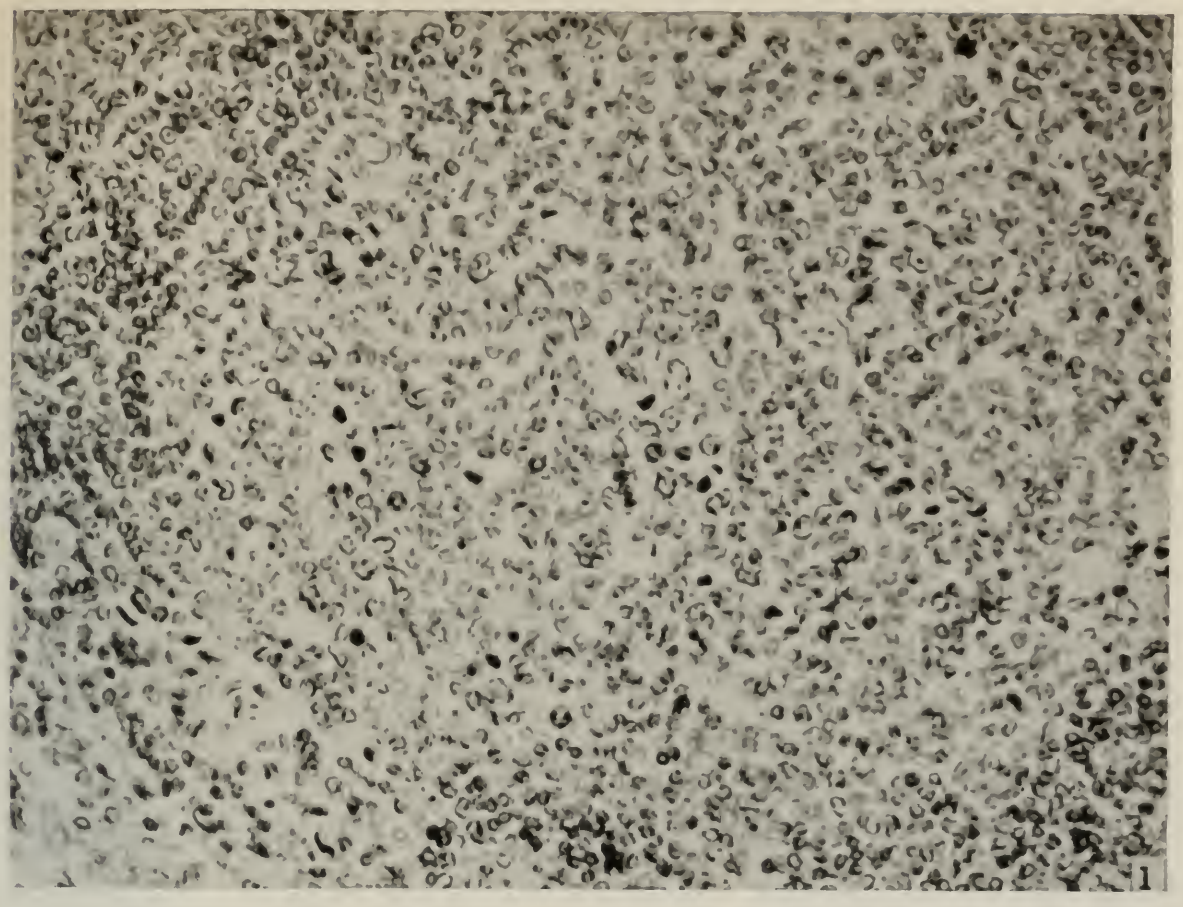



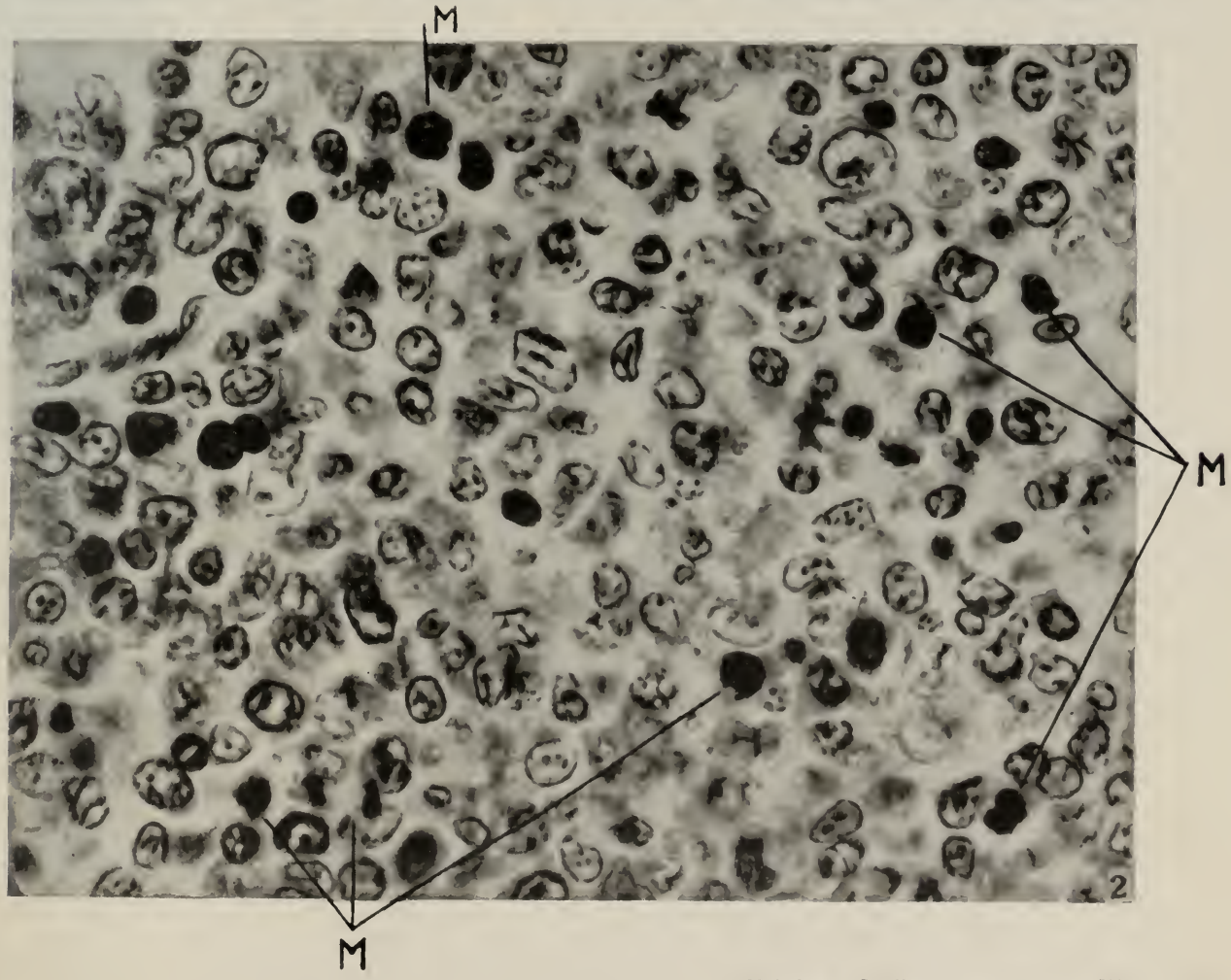

(Nakahara: Lymphocytosis induced by means of heat.



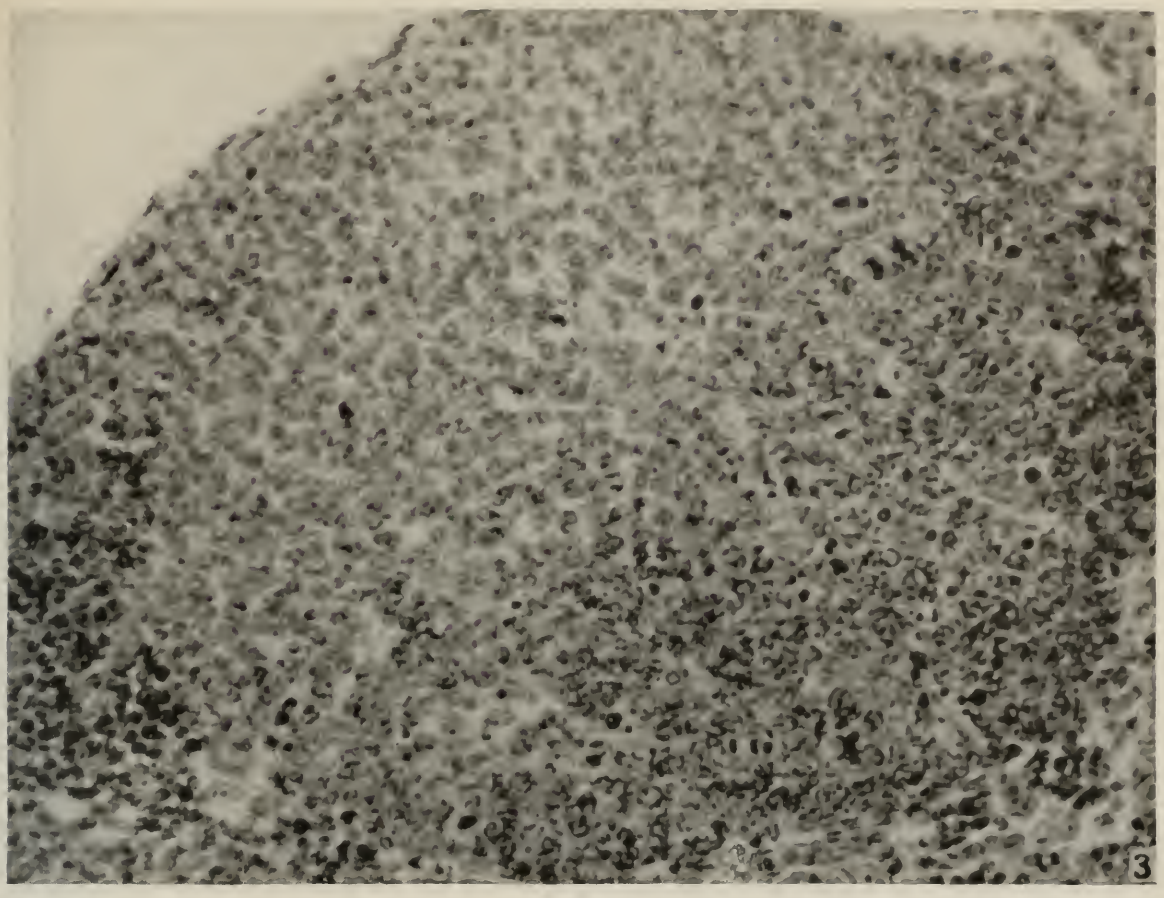



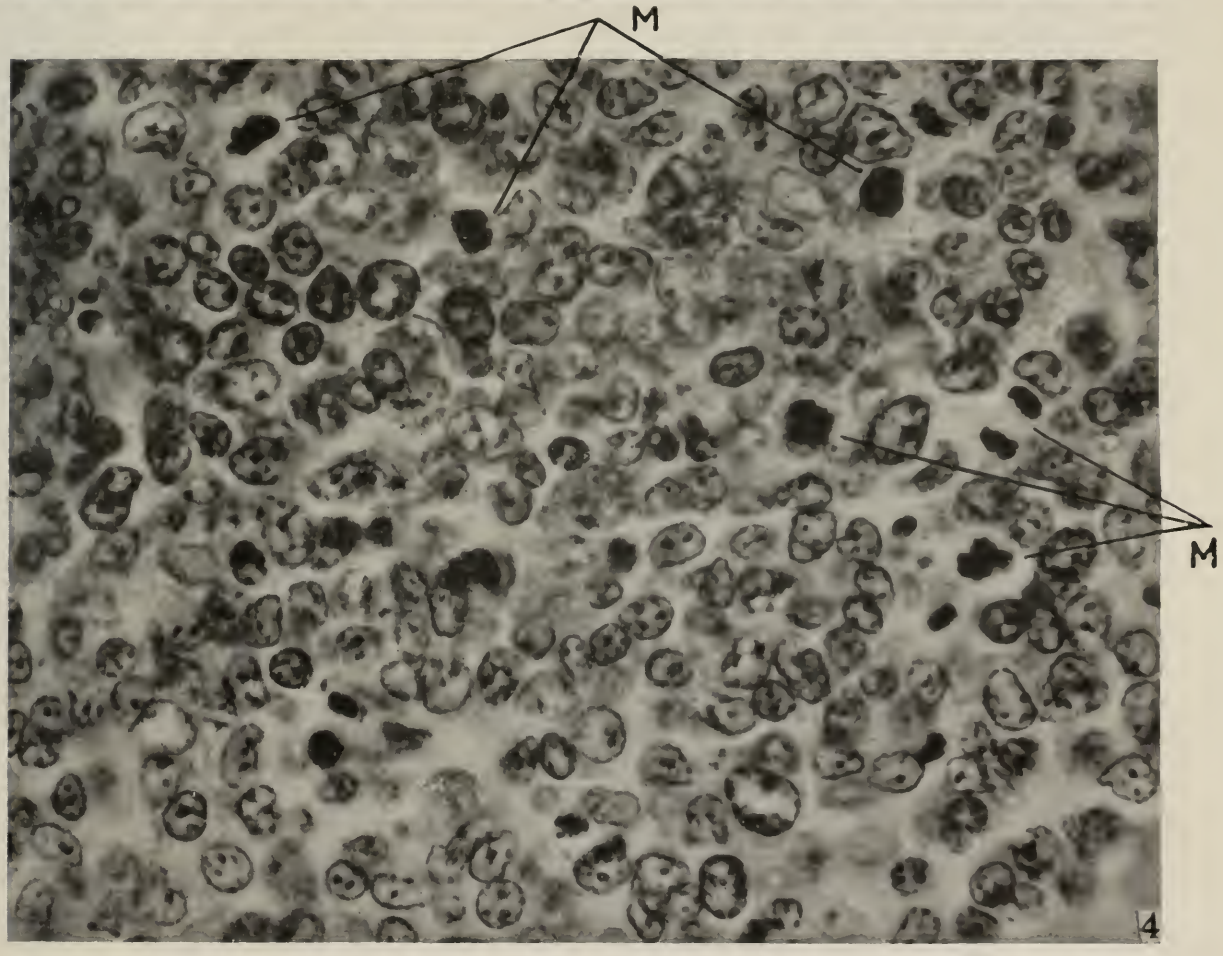

(Nakahara: Lymphocytosis induced by means of heat.)




(Nakahara: Studies on x-ray effects. III.)




(Nakahara: Studies on x-ray effects. III.)

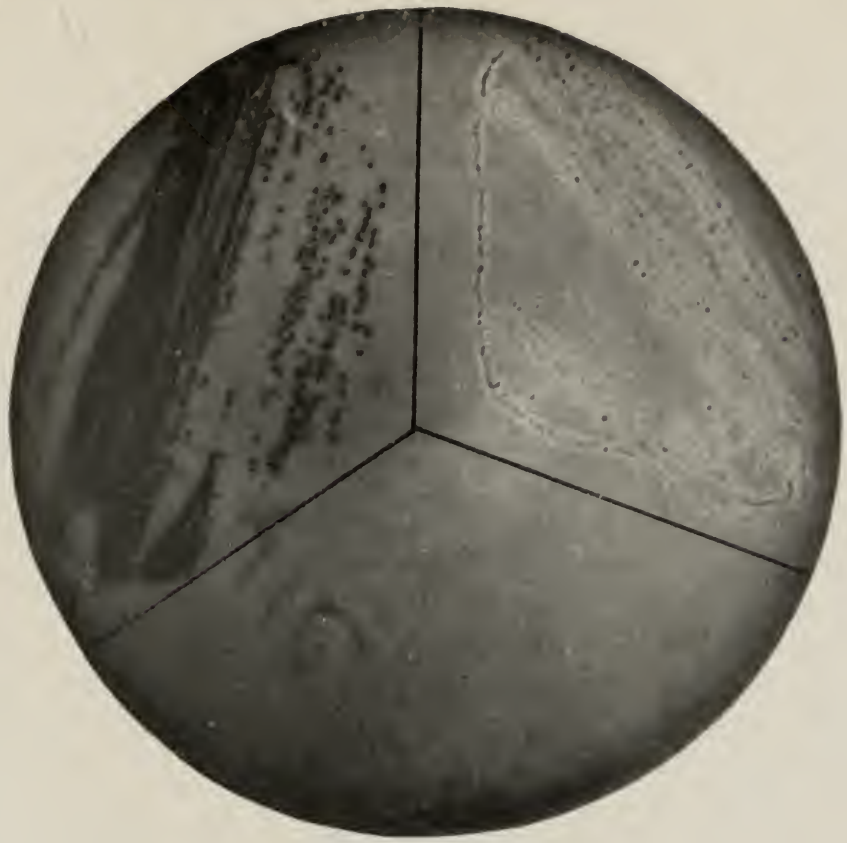


FIG. 1.

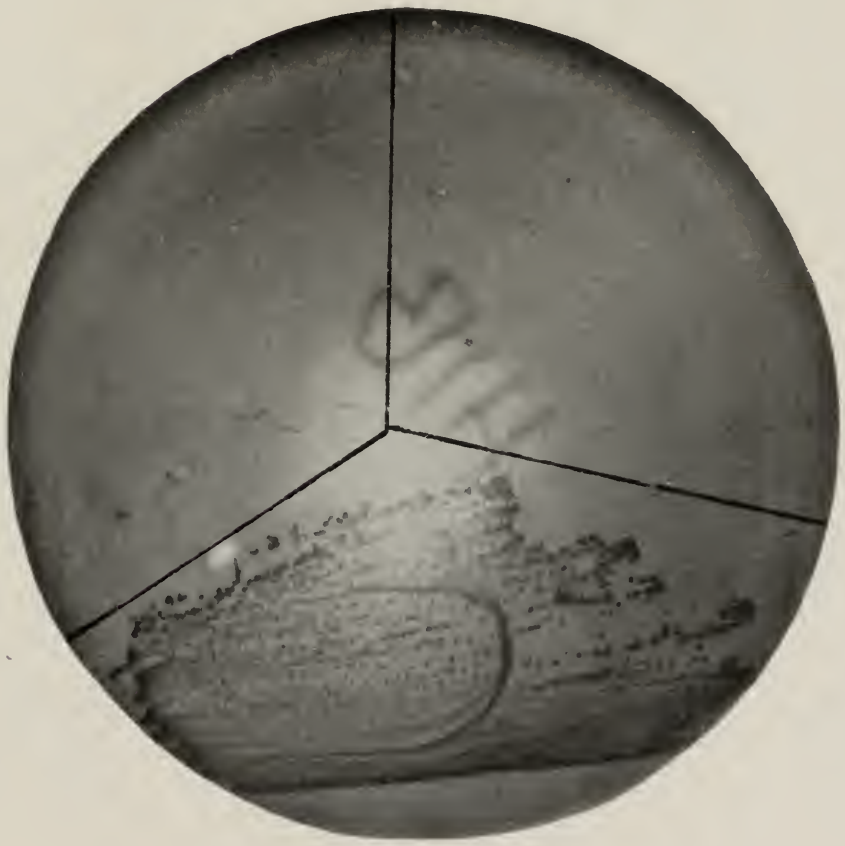


FIG. 2.

(Pritchett and Stillman: *B. influenzæ* in throats and saliva.)



FIG. 3.



FIG. 4.

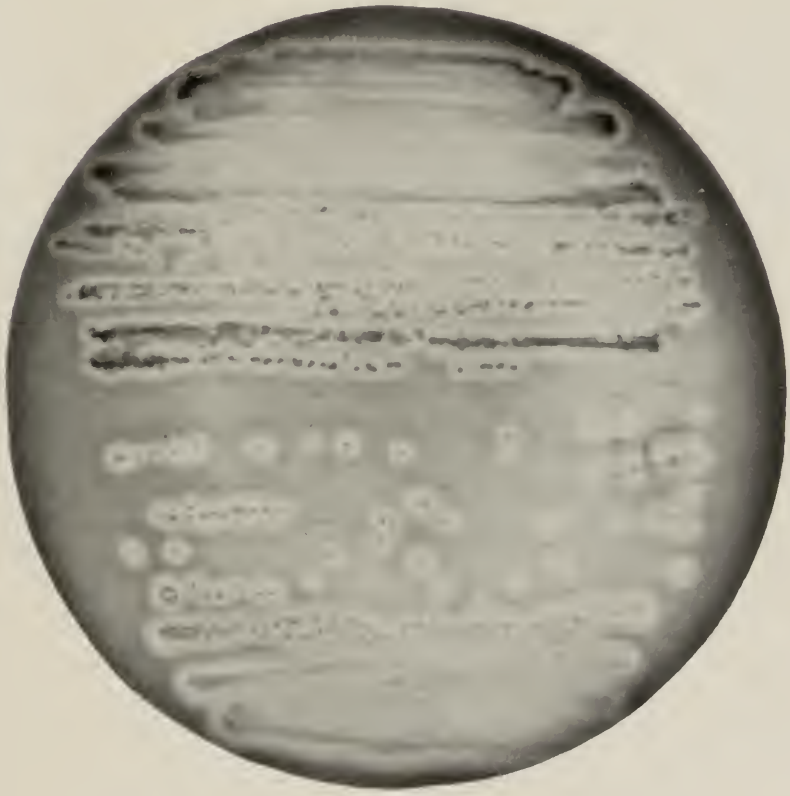


FIG. 5.

(Pritchett and Stillman: *B. influenzæ* in throats and saliva.)

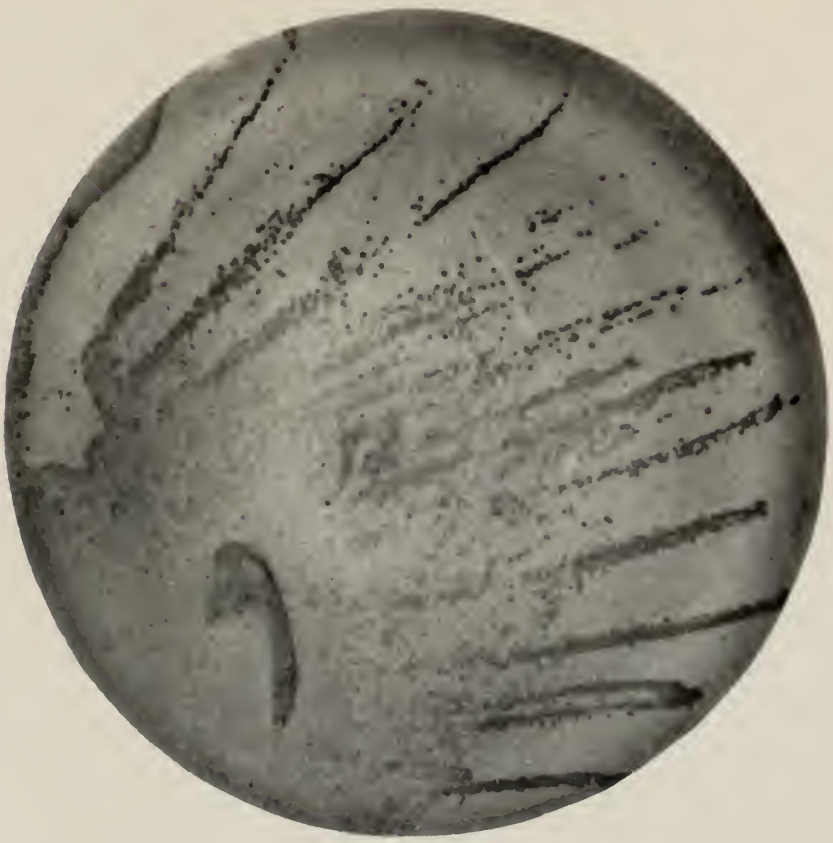


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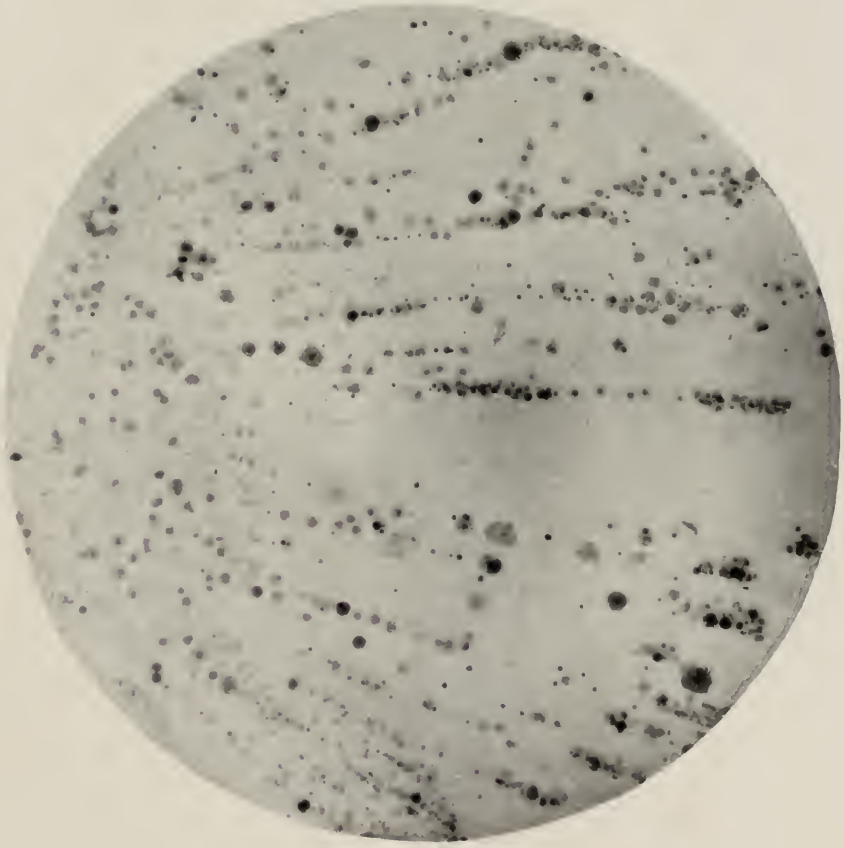


FIG. 7.

(Pritchett and Stillman: *B. influenzæ* in throats and saliva.)

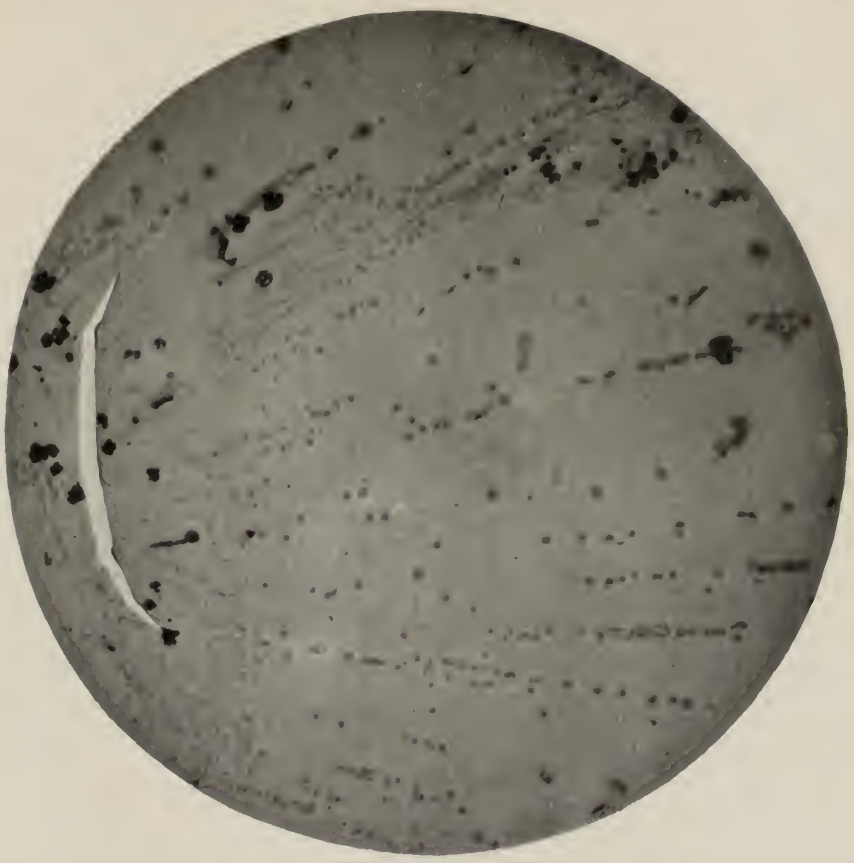


FIG. 8.

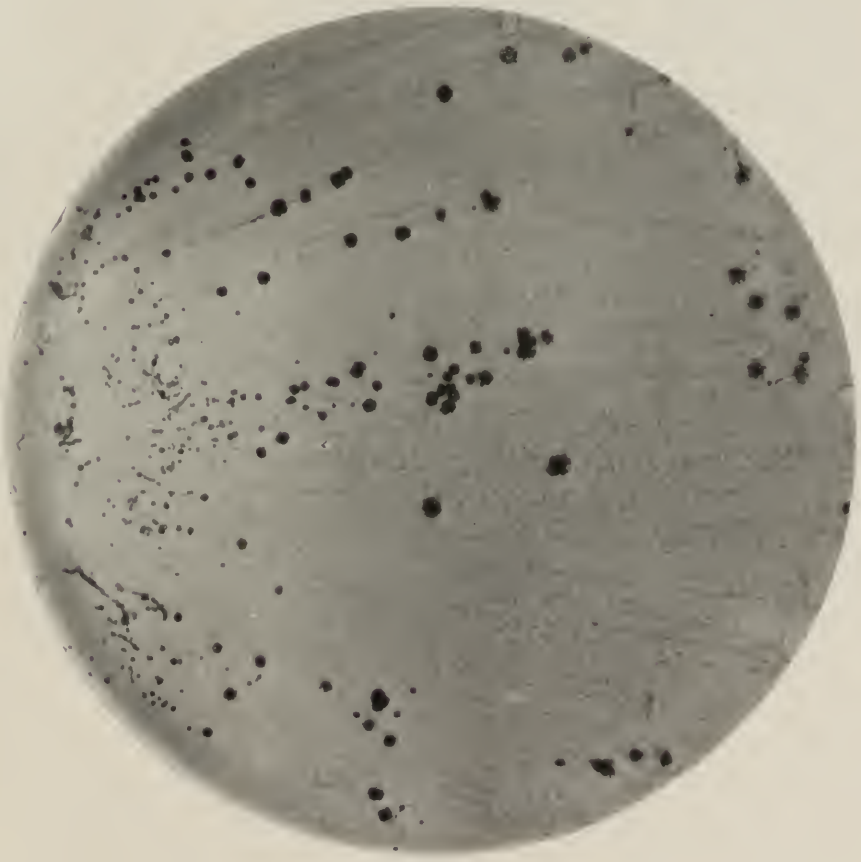


FIG. 9.

(Pritchett and Stillman: *B. influenzæ* in throats and saliva.)

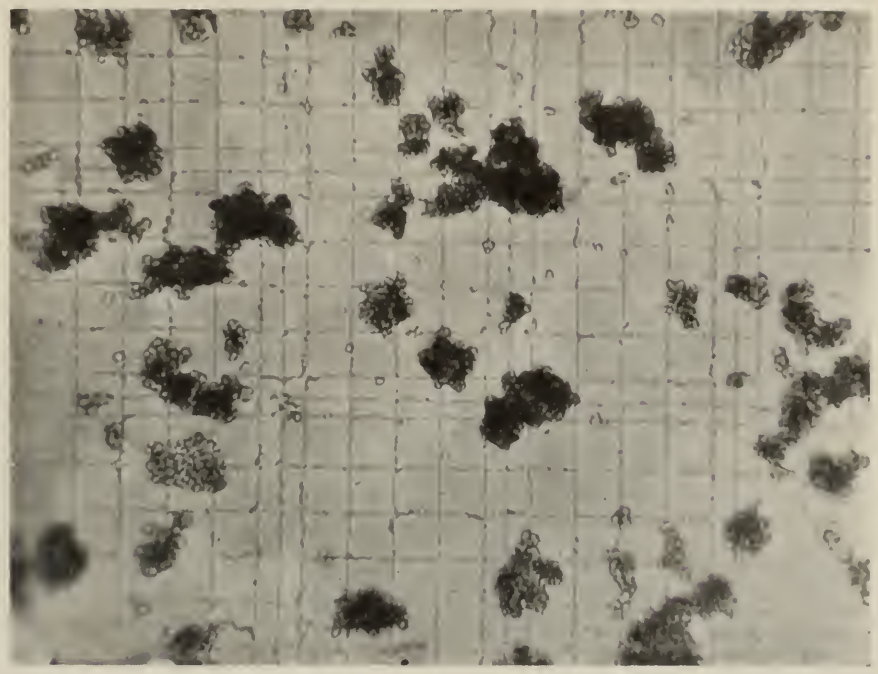


FIG. 1.

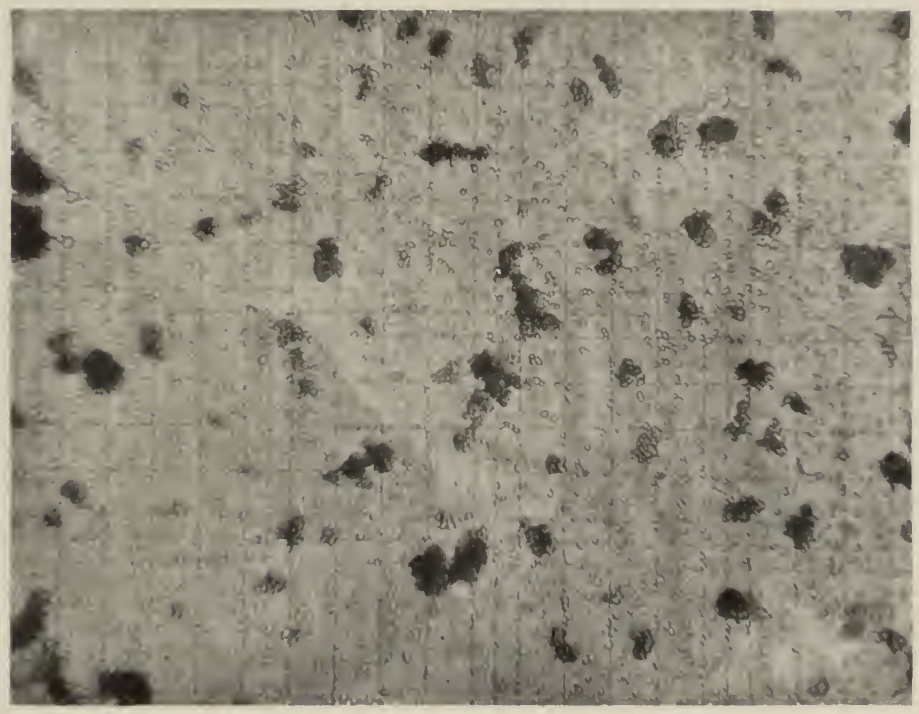
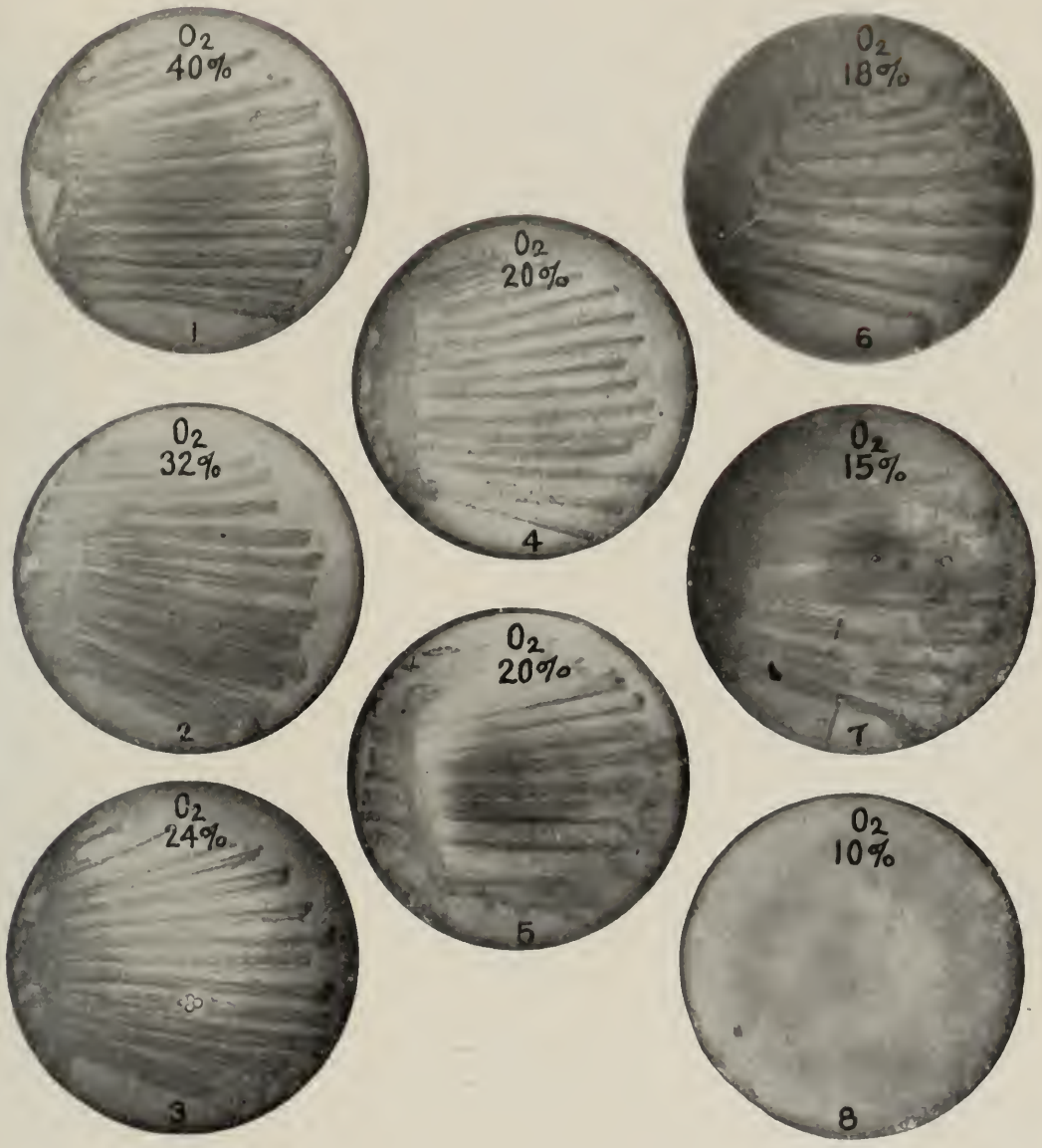
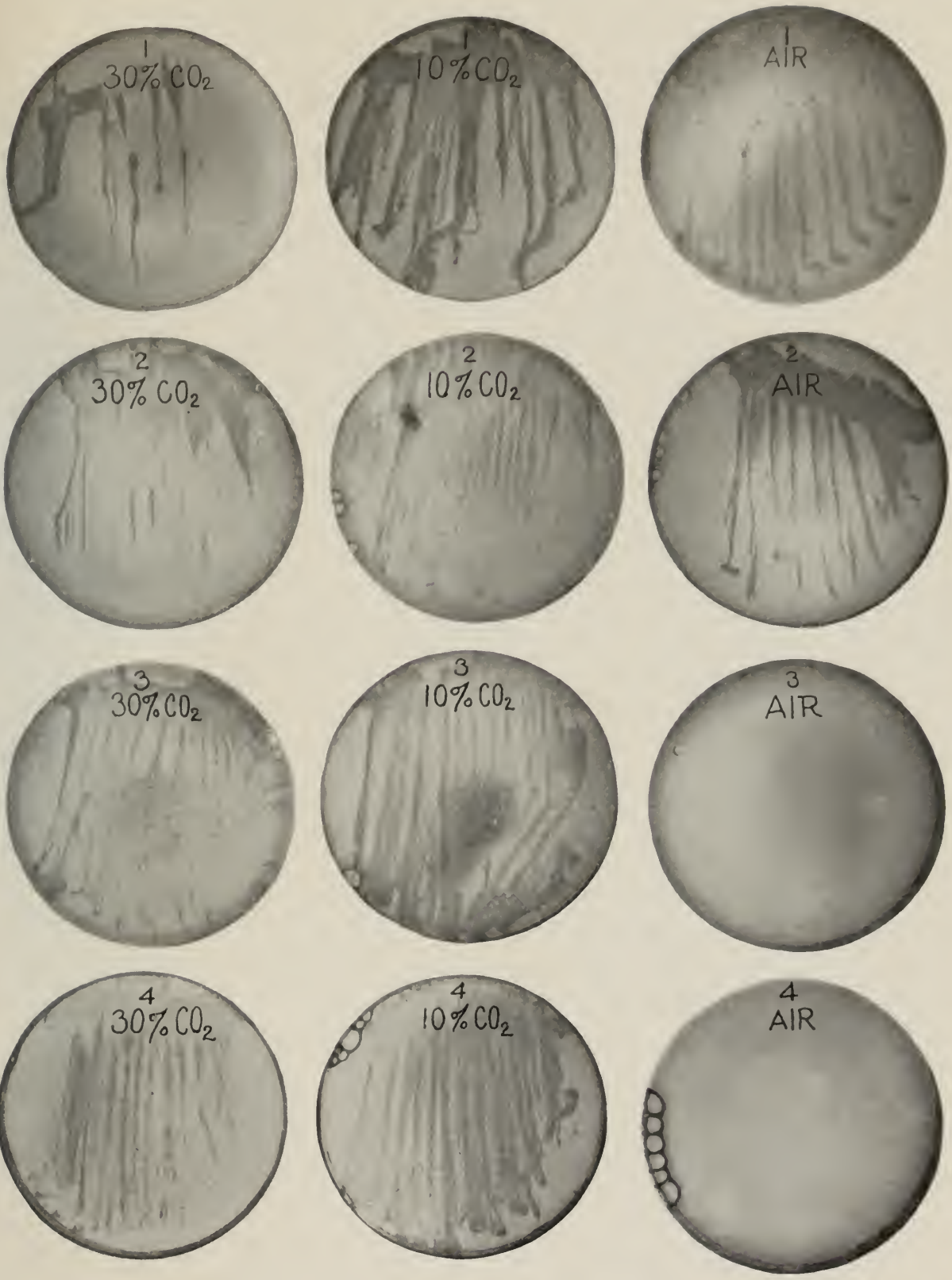


FIG. 2.

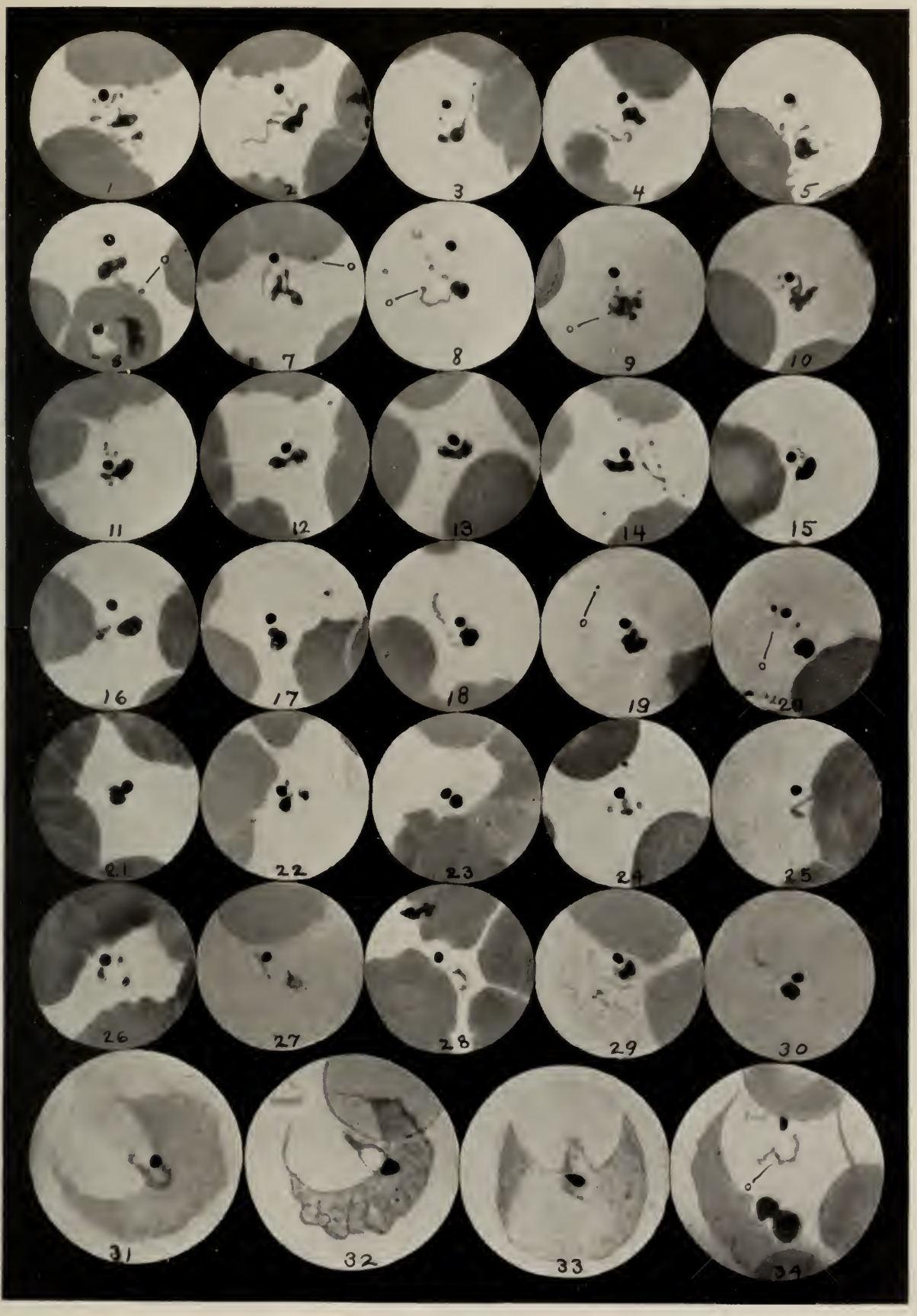
(Ashby: Transfused blood corpuscles.)



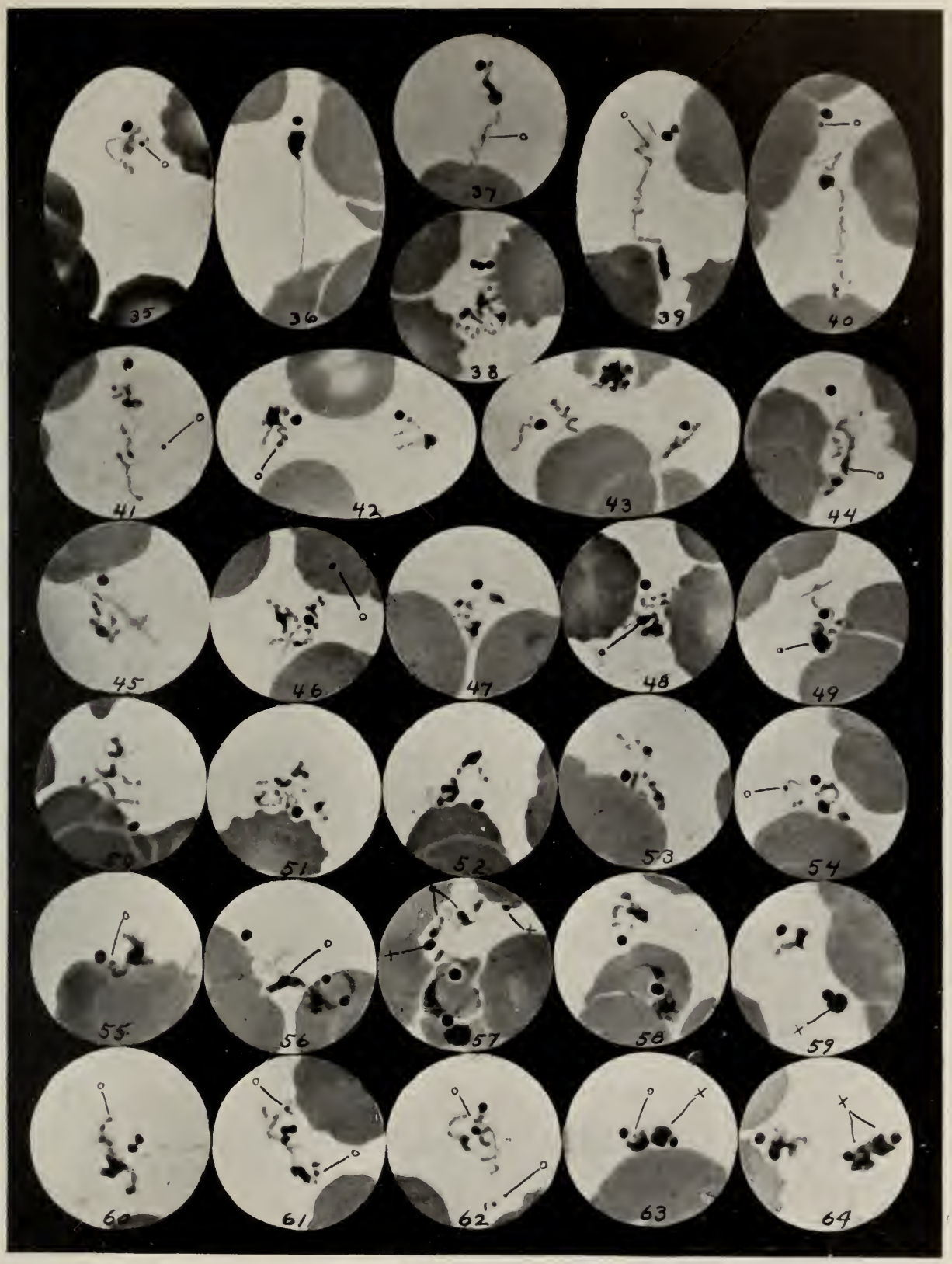

(Gates: Cultivation of meningococcus.)




(Gates: Cultivation of meningococcus.)



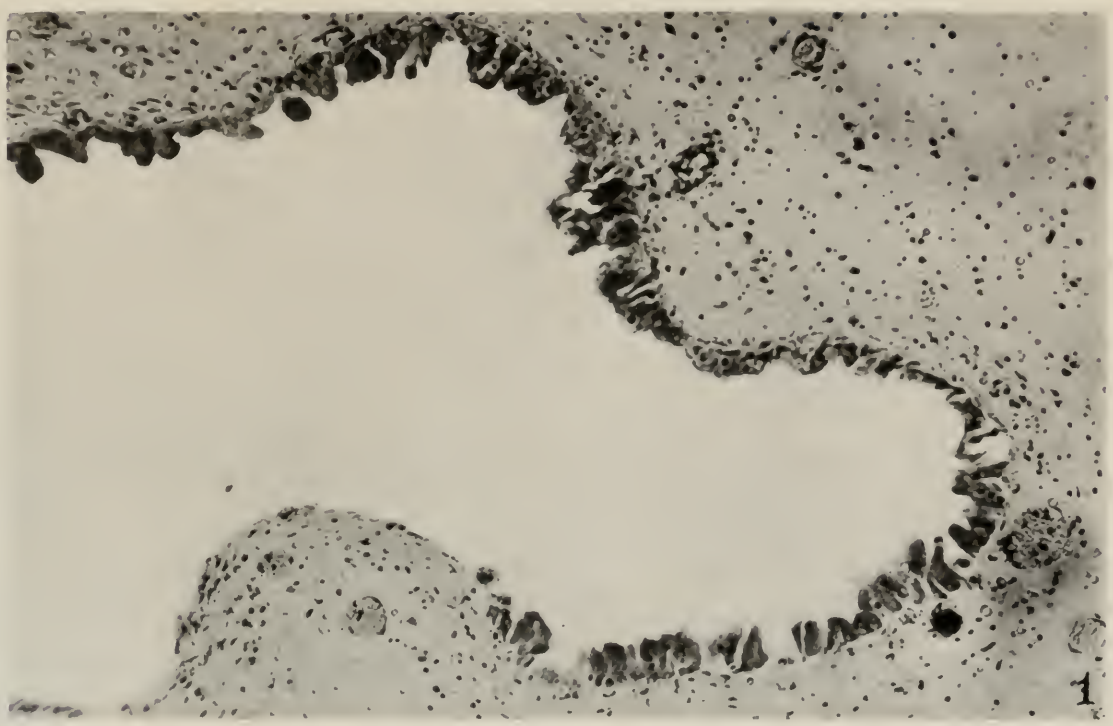

(Lawson: Æstivo-autumnal malarial infections.)

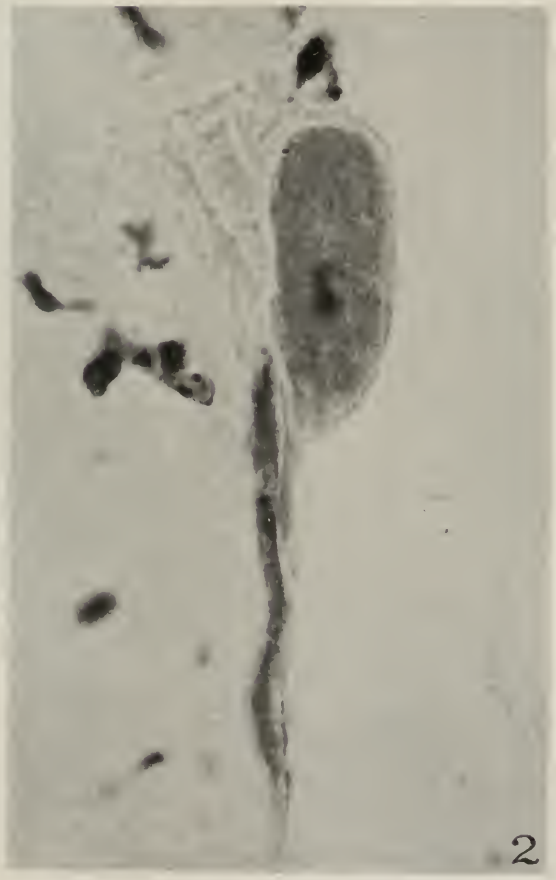


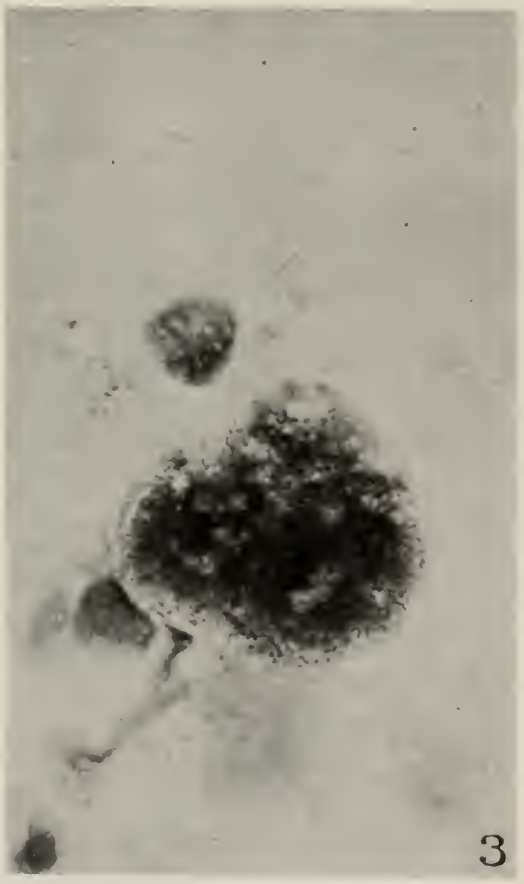

(Lawson: Æstivo-autumnal malarial infections.)



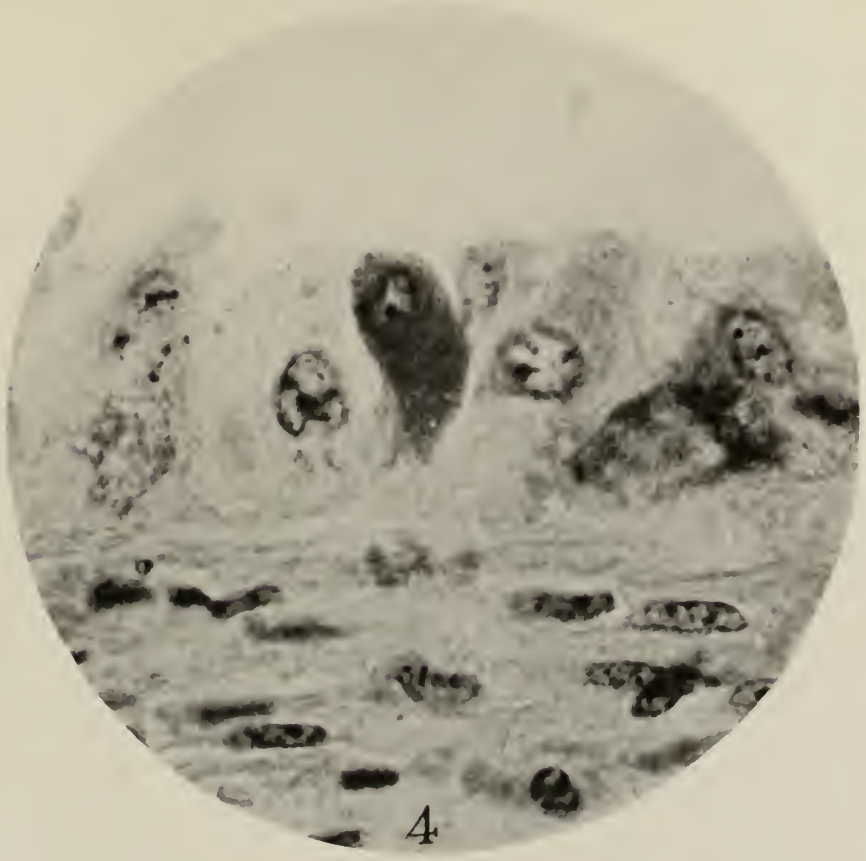

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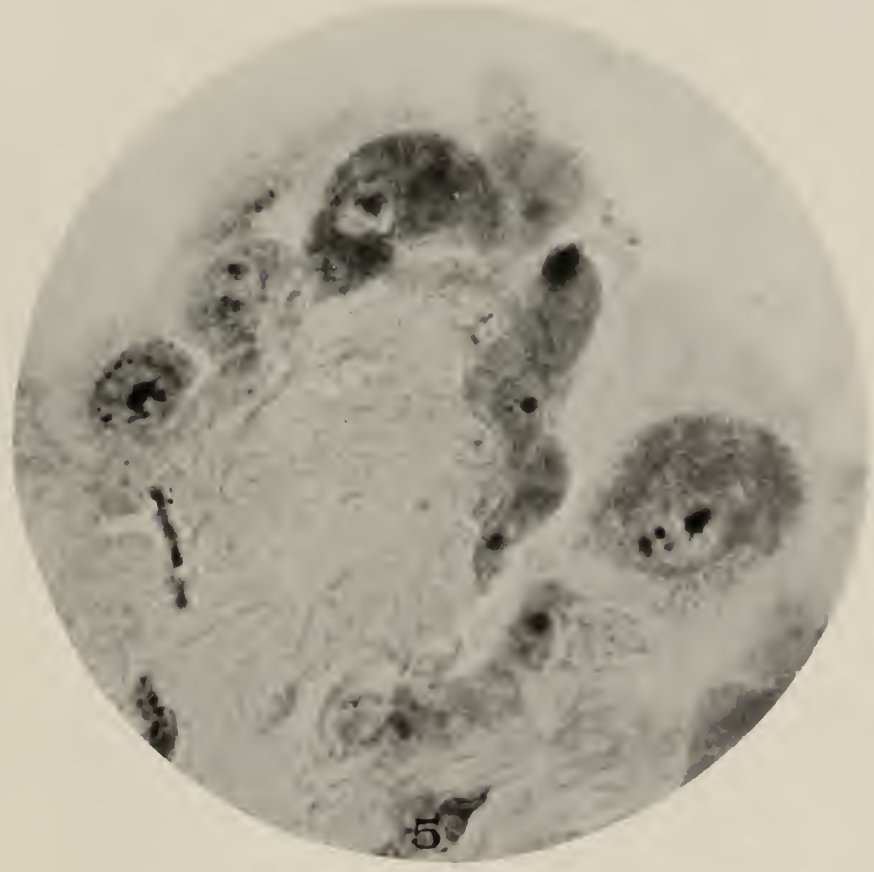


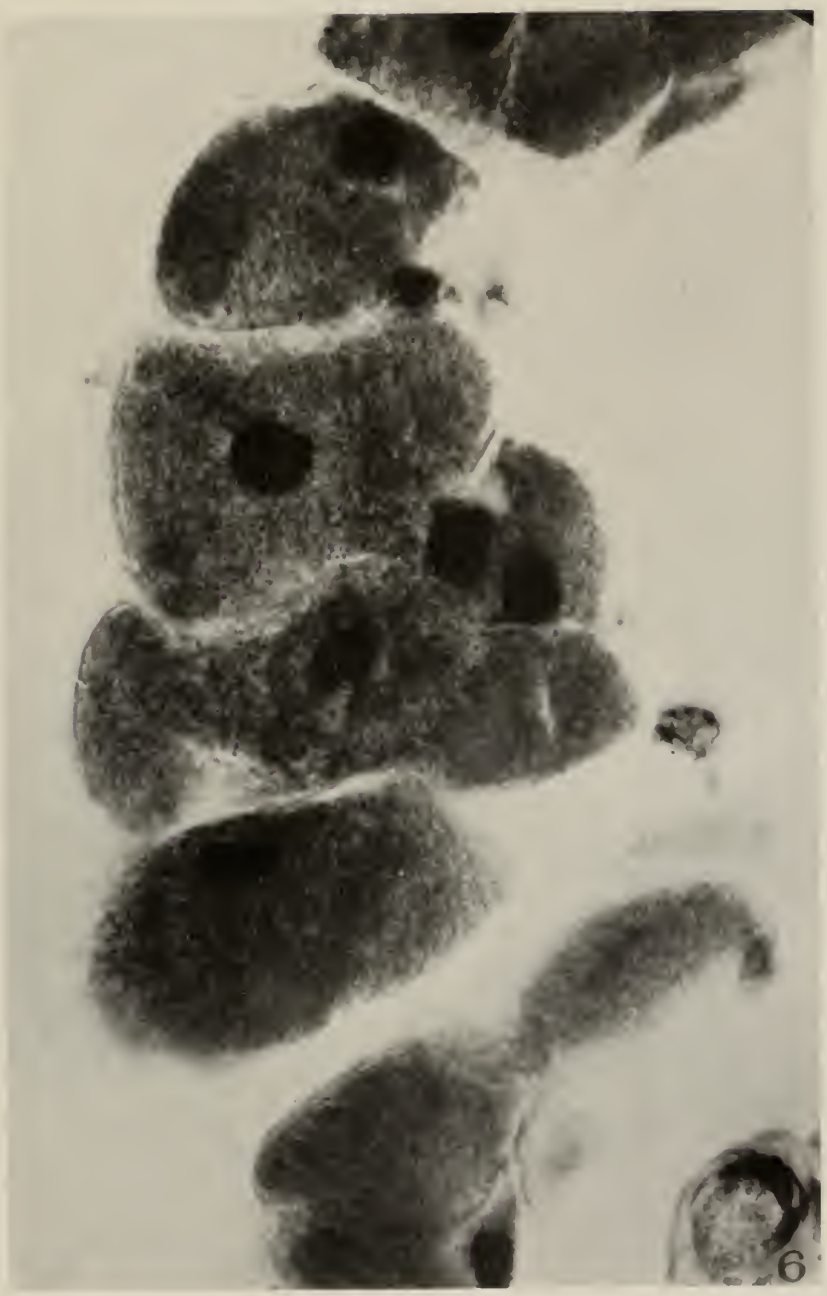




(Smith: Localization of *Bacillus abortus*.)




(Smith: Localization of *Bacillus abortus*.)




(Smith: Localization of *Bacillus abortus*.)

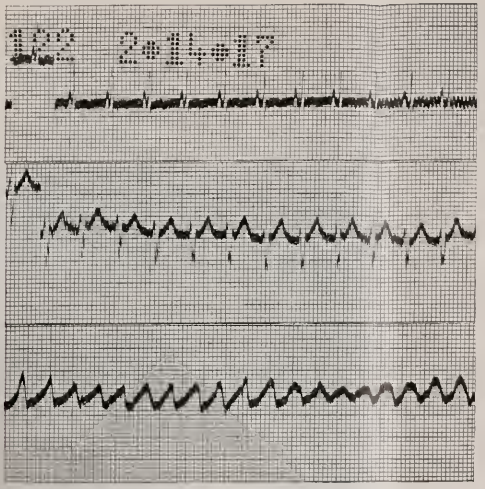


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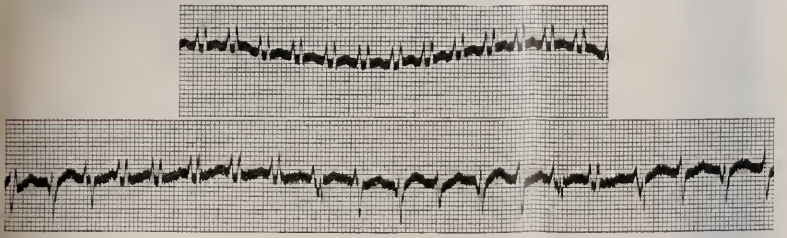
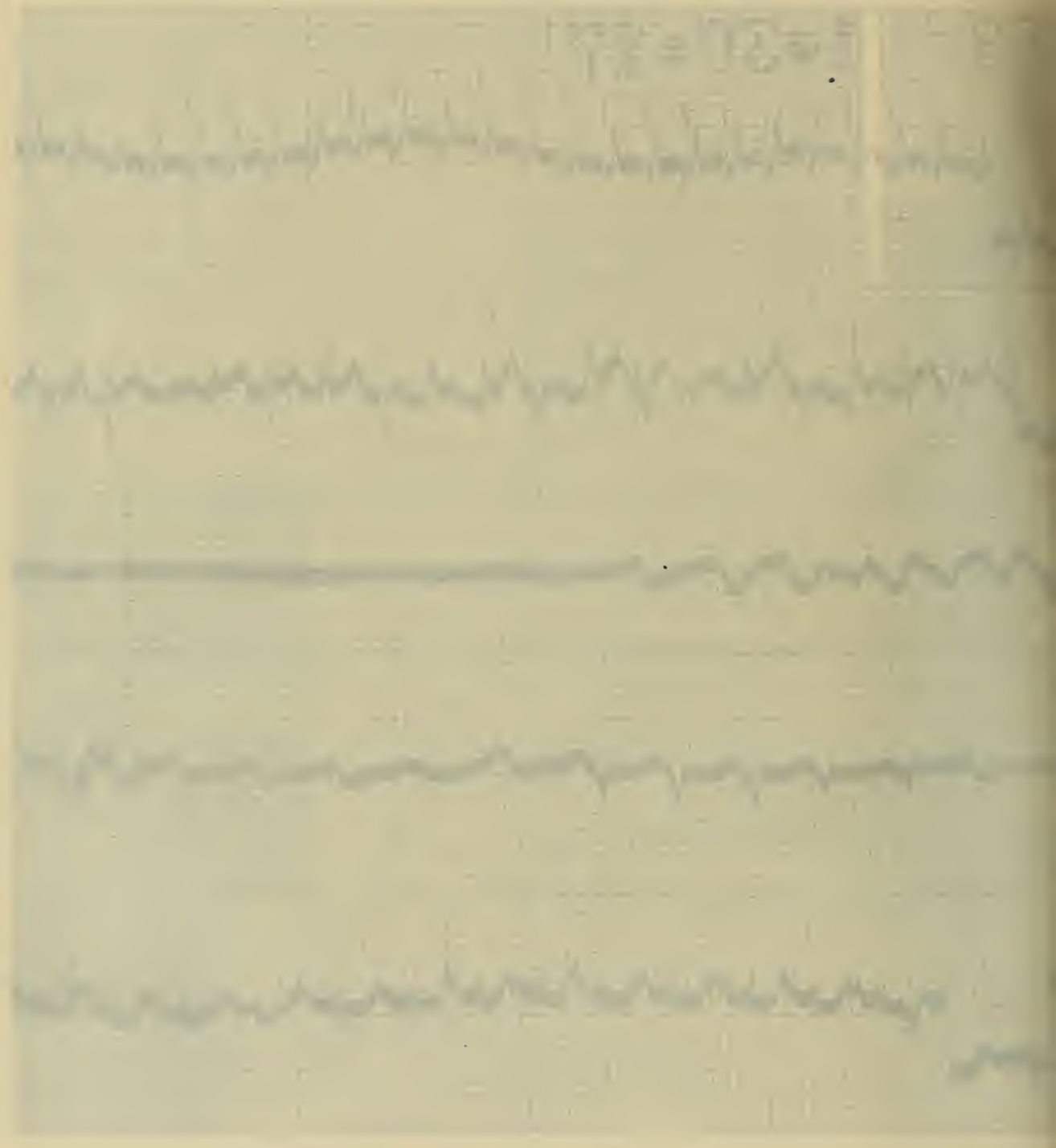


FIG. 2.

(Levine: Action of strophanthin on cat's heart.)



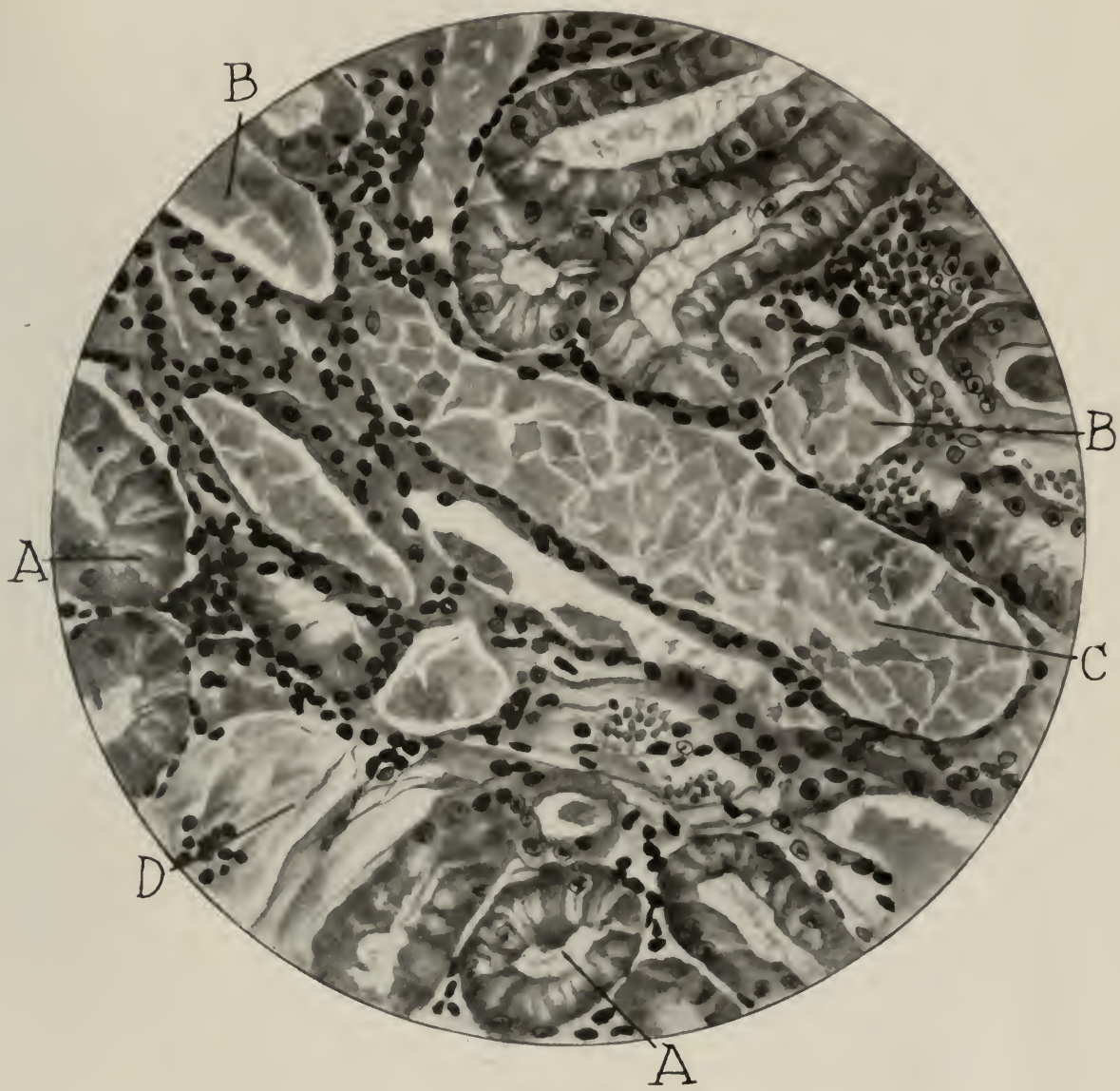



FIG. 1.

(MacNider: Chronic nephropathy by uranium nitrate.)




FIG. 2.

(MacNider: Chronic nephropathy by uranium nitrate.)




FIG. 3.

(MacNider: Chronic nephropathy by uranium nitrate)




FIG. 4.

(MacNider: Chronic nephropathy by uranium nitrate.)

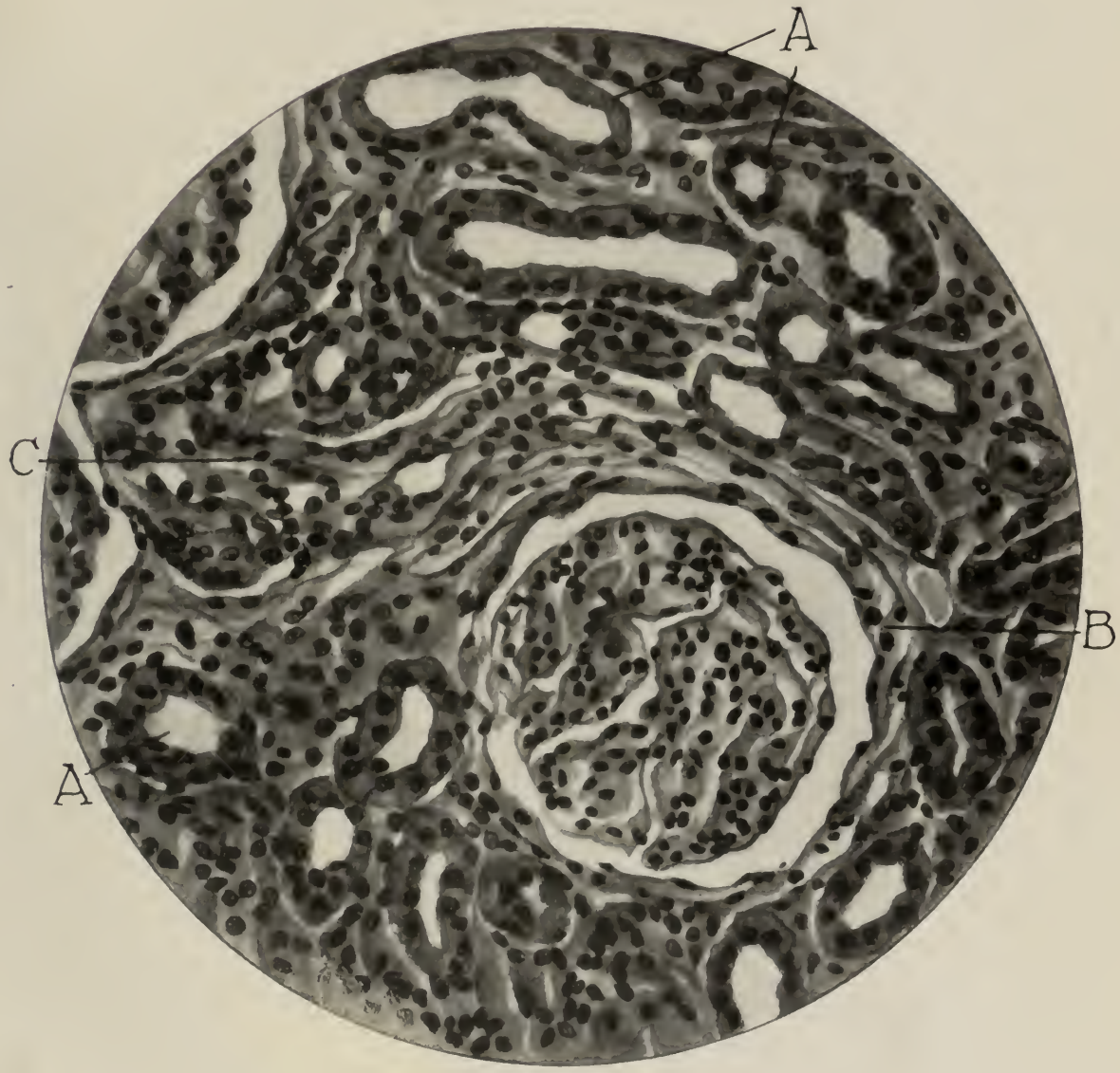



FIG. 5.

(MacNider: Chronic nephropathy by uranium nitrate.)



FIG. 1.



FIG. 2.



FIG. 3.

(Noguchi: Etiology of yellow fever. I.)

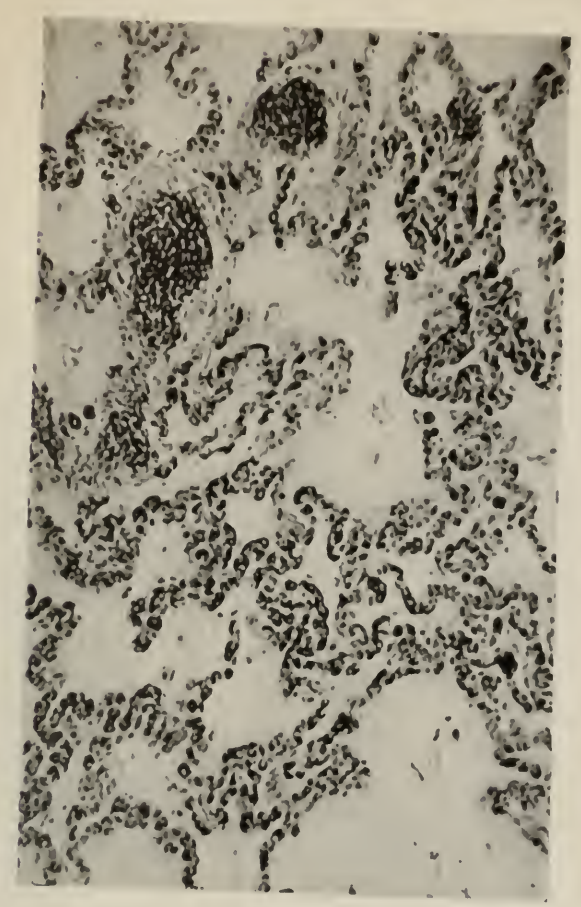


FIG. 4.

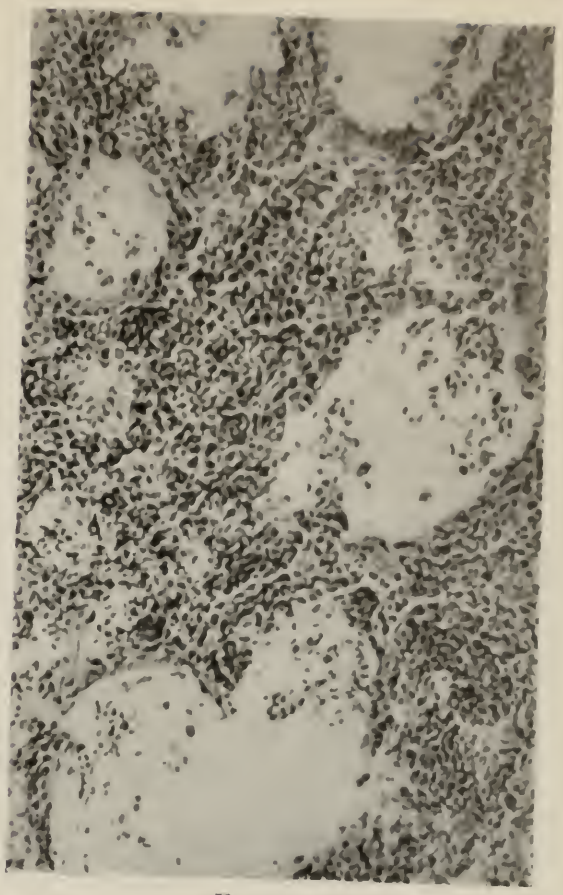


FIG. 6.

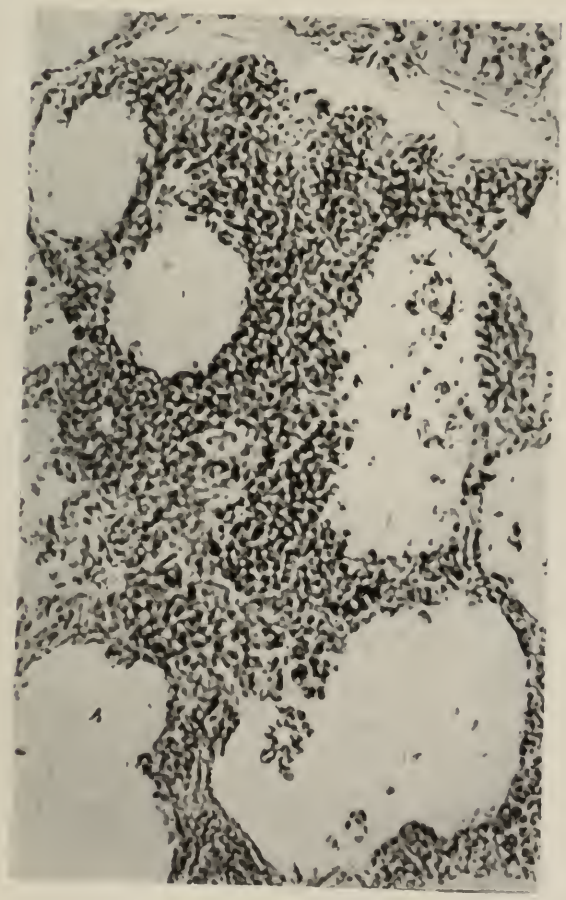


FIG. 5.

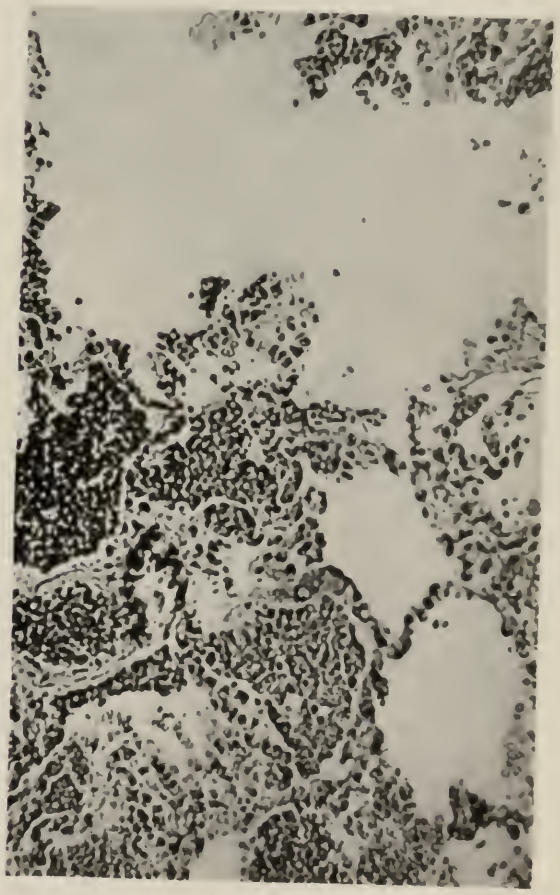


FIG. 7.

(Noguchi: Etiology of yellow fever. I.)

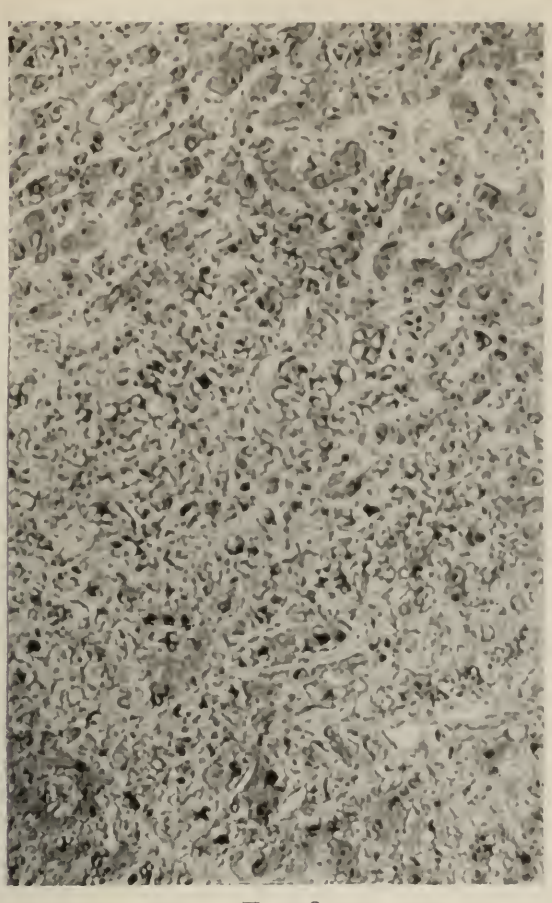


FIG. 8.



FIG. 10.

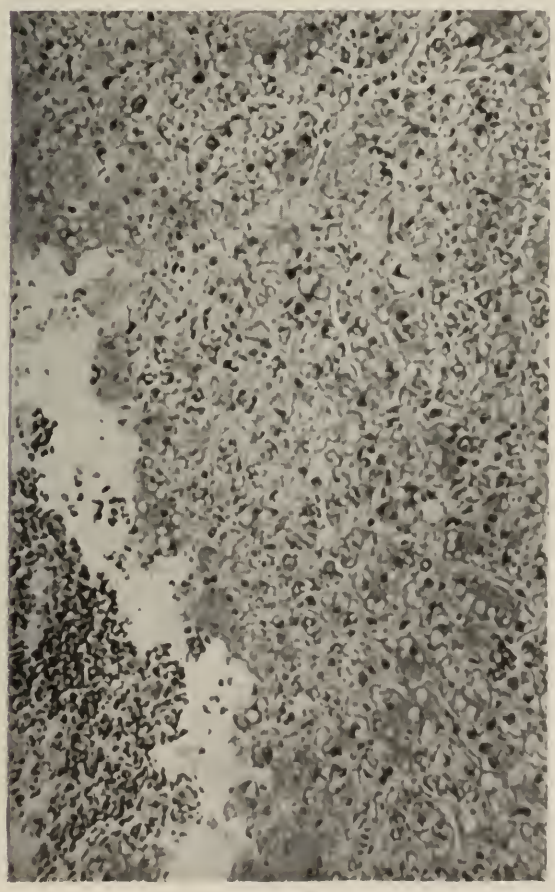


FIG. 9.



FIG. 11.

(Noguchi: Etiology of yellow fever. I.)

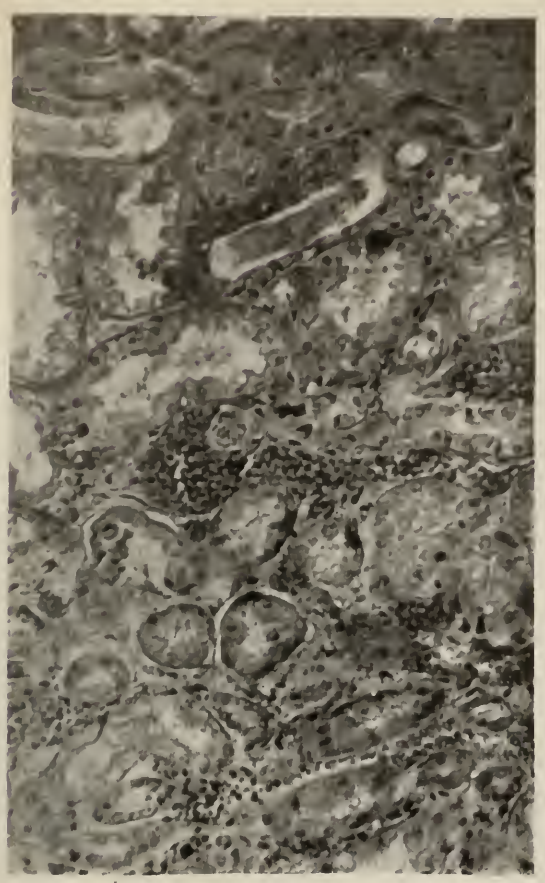


FIG. 12.

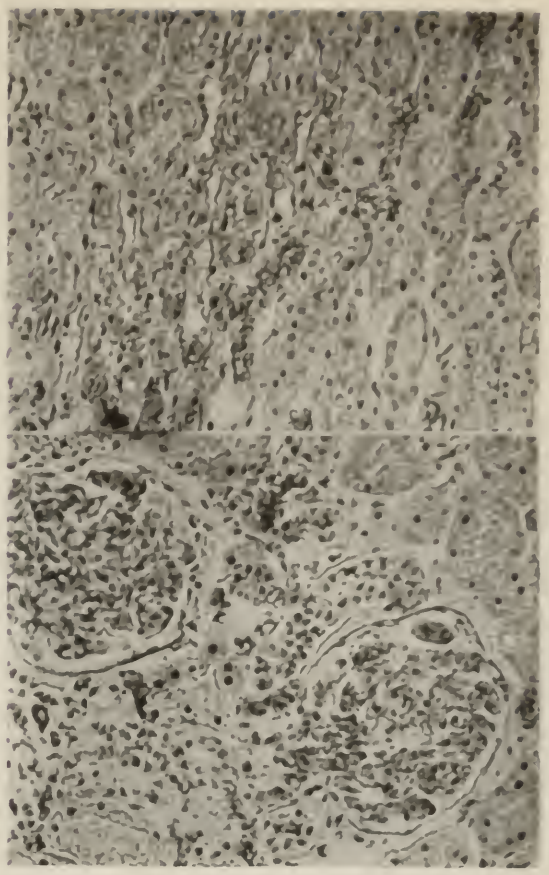


FIG. 14.

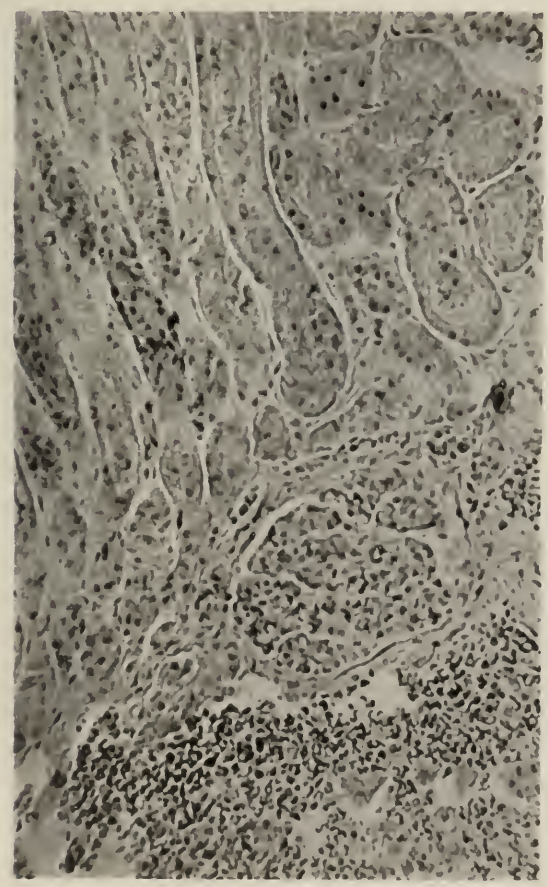


FIG. 13.

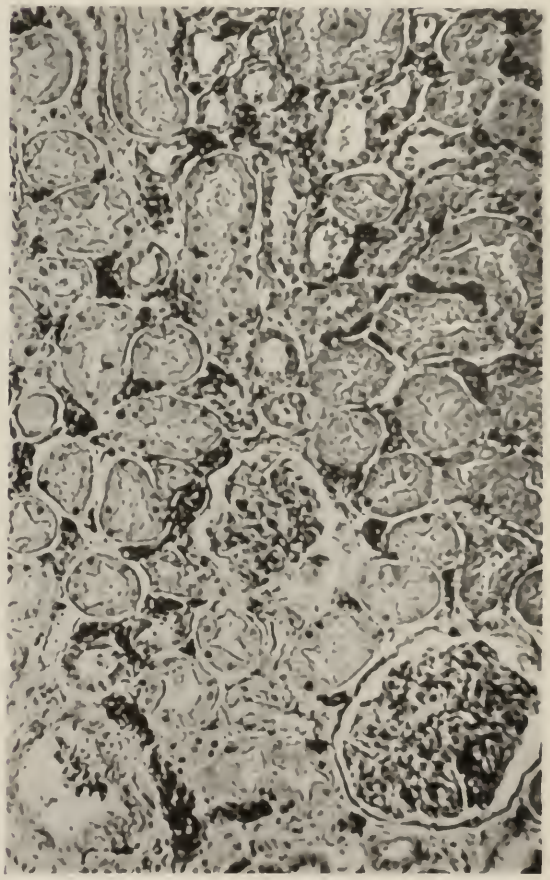


FIG. 15.

(Noguchi; Etiology of yellow fever. I.)

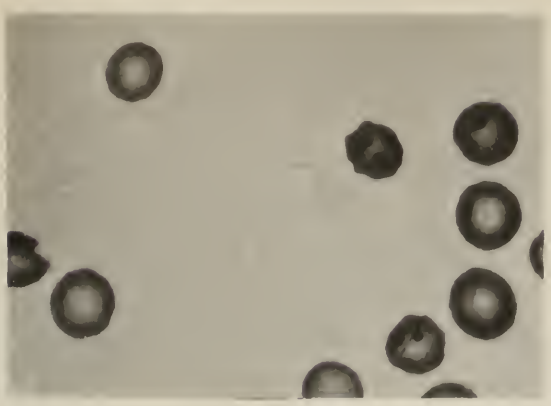


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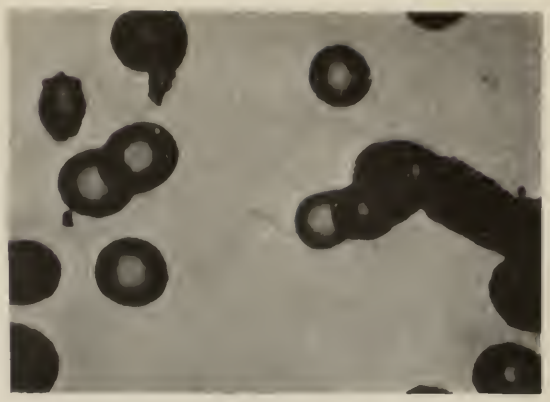


FIG. 2.

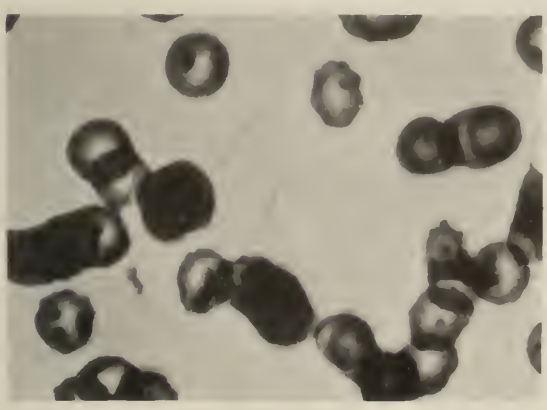


FIG. 3.

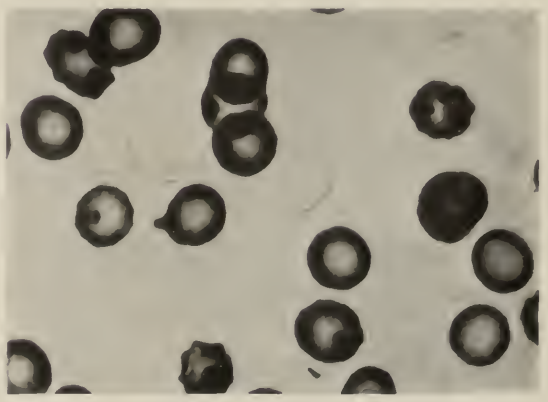


FIG. 4.



FIG. 5.



FIG. 6.

(Noguchi: Etiology of yellow fever. II.)

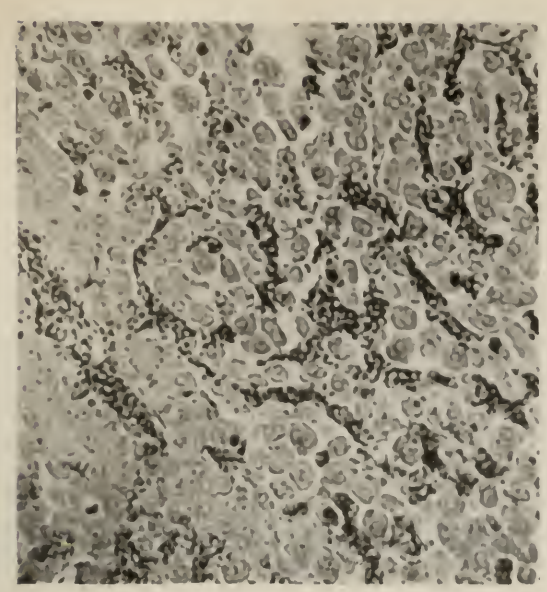


FIG. 1.



FIG. 4.

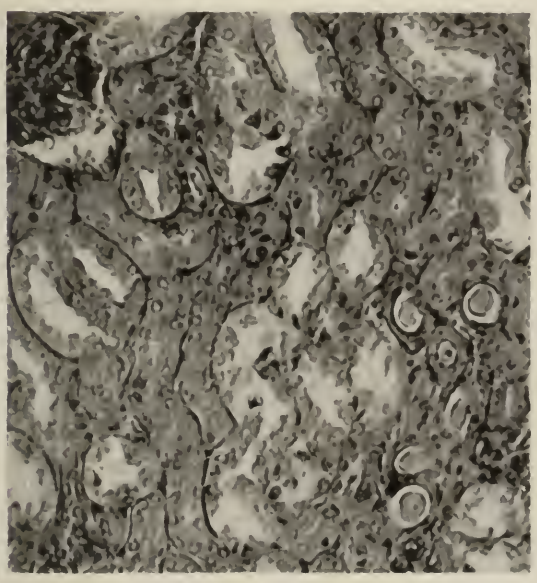


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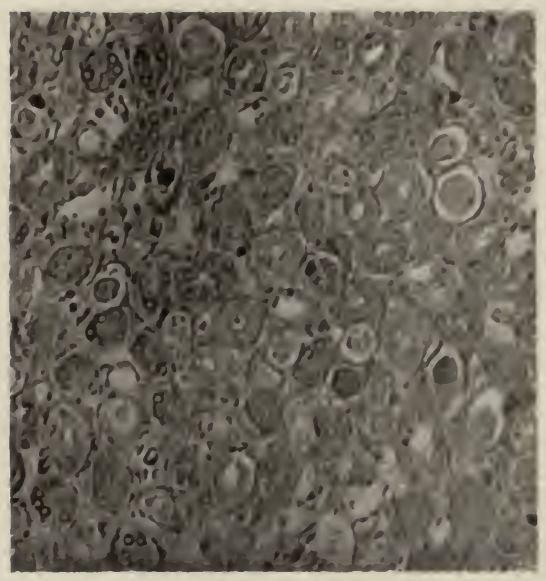


FIG. 5.

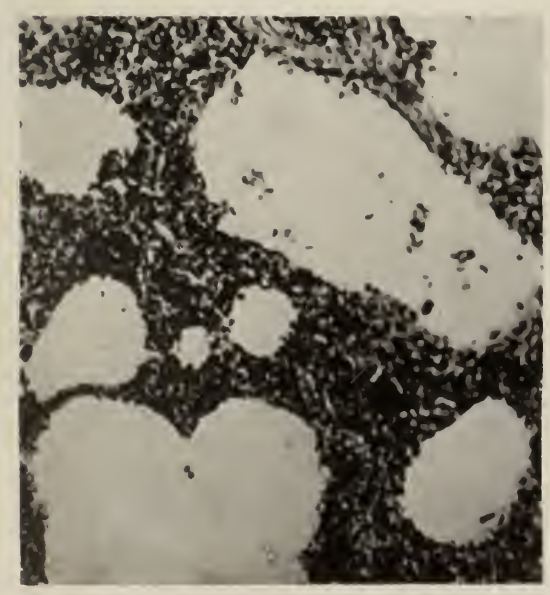


FIG. 3.

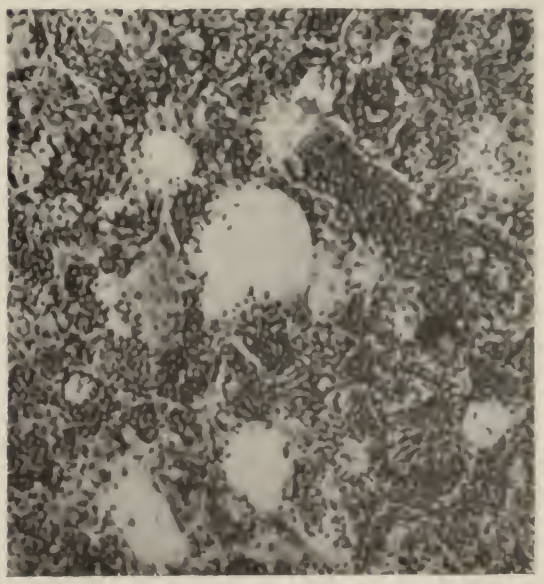


FIG. 6.

(Noguchi: Etiology of yellow fever. III.)



FIG. 7.

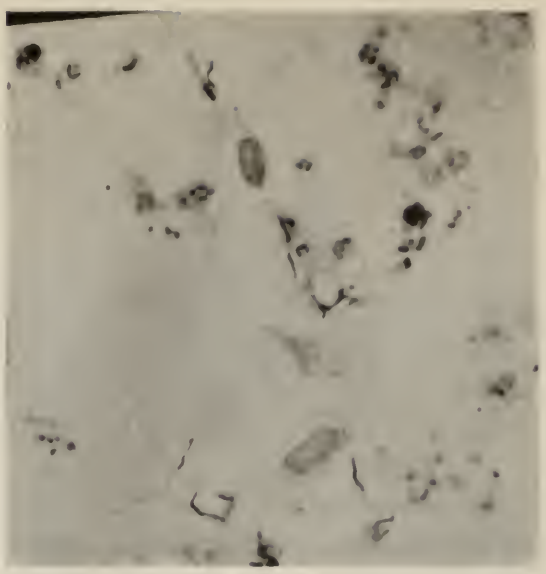


FIG. 10.

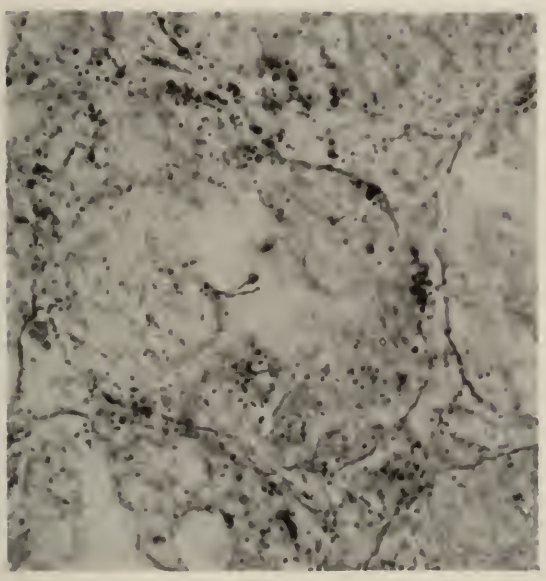


FIG. 8.

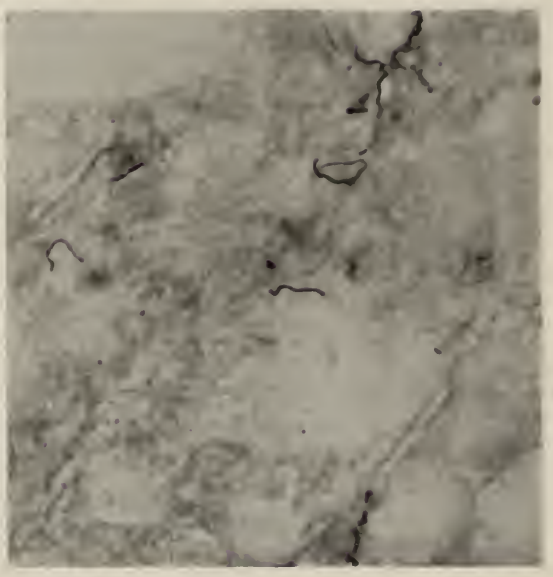


FIG. 11.



FIG. 9.



FIG. 12.



FIG. 13.



FIG. 14.

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OF

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EDITED BY

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VOLUME XXIX, NO. 6 JUNE 1, 1919



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